



University of Connecticut
OpenCommons@UConn

Master's Theses

University of Connecticut Graduate School

4-22-2014

Efficacy of Plant Compounds for Reducing Foodborne Pathogens on Fresh Produce

Tyler E. Mattson

University of Connecticut - Storrs, tyler.mattson51@yahoo.com

Recommended Citation

Mattson, Tyler E., "Efficacy of Plant Compounds for Reducing Foodborne Pathogens on Fresh Produce" (2014). *Master's Theses*. 551.
https://opencommons.uconn.edu/gs_theses/551

This work is brought to you for free and open access by the University of Connecticut Graduate School at OpenCommons@UConn. It has been accepted for inclusion in Master's Theses by an authorized administrator of OpenCommons@UConn. For more information, please contact opencommons@uconn.edu.

Efficacy of Plant Compounds for Reducing Foodborne Pathogens on Fresh Produce

Tyler Evan Mattson

B.S., University of Connecticut, 2006

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

At the

University of Connecticut

2014

Copyright by

Tyler Evan Mattson

2014

Tyler Evan Mattson

APPROVAL PAGE

Masters of Science Thesis

Efficacy of Plant Compounds for Reducing Foodborne Pathogens on Fresh Produce

Presented by

Tyler Evan Mattson, B.S.

Major
Advisor_____

Kumar Venkitanarayanan

Associate
Advisor_____

Cameron L. Faustman

Associate
Advisor_____

Thomas A. Hoagland

University of Connecticut

2014

Acknowledgements

I would like to thank my major advisor Dr. Kumar Venkitanarayanan for everything he has taught me and for all of his guidance. Thank you to my committee members Dr. Cameron Faustman and Dr. Thomas Hoagland. I am very grateful to my lab mate Anup Kollanoor Johny for all of his guidance and friendship. I would like to thank Karen More and David T. Schreiber for all of their technical support. I would lastly like to thank my wonderful lab mates Mary Anne Roshni Amalaradjou, Sangeetha Baskaran, and Anu Susan Charles, without all of you this wouldn't have been possible.

Table of contents

Abstract.....	v
Chapter 1: Introduction	1
Chapter 2: Review of Literature	5
Current trends in fresh produce production and consumption	6
Foodborne illness burden in the United States	7
Contamination of fresh produce.....	8
Escherichia coli O157:H7	9
Listeria monocytogenes.....	9
Salmonella spp.	10
Outbreaks linked to fresh produce (Table 1).....	11
Methods for reducing pathogens on fresh produce	14
Chlorine.....	14
Toxicity of chlorine compounds	15
Peroxyacetic acid.....	16
Hydrogen peroxide.....	16
Ozone	17
Natural alternatives	17
Antimicrobial mechanisms of plant molecules	18
Chapter 3: Inactivation of <i>Salmonella</i> spp. on tomatoes by plant molecules	36
Chapter 4: Effect of <i>trans</i>-cinnamaldehyde and carvacrol on <i>Escherichia coli</i>	
<i>O157:H7</i> and <i>Listeria monocytogenes</i> on iceberg lettuce	61
Chapter 5: Conclusion.....	91

Abstract

Four plant derived compounds, namely carvacrol (CAR), *trans*-cinnamaldehyde (TC), eugenol (EUG) and β -resorcylic acid (BR) were investigated as wash treatments for reducing *Salmonella* spp. on tomatoes. In addition, the efficacy of TC and CAR in inactivating *E. coli* O157:H7 and *L. monocytogenes* on iceberg lettuce in the presence and absence of 10% organic matter was studied. Results suggest that CAR, TC, EUG and BR were effective in rapidly reducing large populations of *Salmonella* spp. on tomatoes when used in wash water, and CAR rapidly decreased *E. coli* O157: H7 and *L. monocytogenes* populations on lettuce even in the presence of organic matter. The efficacy of aforementioned plant compounds for decontaminating fresh produce should be validated under commercial settings. In addition, extensive sensory and storage studies of fresh produce treated with plant compounds are warranted before recommending their usage in the produce industry.

Chapter 1: Introduction

The per capita consumption of fresh produce has been steadily increasing during the last decade in the United States (Beuchat et al., 1997; Pollack, 2001; U.S. General Accounting Office, 2002). However, fresh produce has also been increasingly associated with foodborne disease outbreaks. The proportion of outbreaks linked to fresh produce increased from less than 1% of all reported outbreaks with a known food vehicle in the 1970s to 6% in the 1990s (Sivapalasingam et al., 2004). Changes in handling and production practices, increased consumption of fresh and minimally processed fruits and vegetables, and increased importation of produce from different regions or countries where standards for production are compromised (Beuchat, 1996; Olaimat et al., 2012) are attributed as the contributing factors to the increased produce-borne outbreaks nationally.

There are many potential sources of contamination of fresh produce from farm-to-fork. These sources include: soil, feces, irrigation water, green or inadequately composted manure, air (dust), wild and domestic animals, human handling, harvesting equipment, transport containers, wash and rinse water, processing equipment, ice, transport vehicles, improper storage, improper packaging, cross-contamination with other foods in storage or display areas, and improper handling after wholesale or retail purchase (Beuchat, 1996). Due to these manifold sources, there is high likelihood that several pathogens, including *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella spp.*, *Shigella spp.* and the Hepatitis A Virus (Davis et al., 1988; Farber et al., 1990; CDC, 1997a & 1997b) are encountered in the produce sold to consumers.

The most commonly used method to remove or inactivate pathogens from fruit and vegetable surfaces is by washing with sodium-chlorinated water (NaClO) (Gunduz et

al., 2009). Chlorine wash systems (most often water flumes) are used by numerous (76%) fresh produce manufacturers for decontamination (Gunduz et al., 2009; Zhang et al., 2009). Many studies have shown that even when produce is washed with 50-200 ppm chlorine solution; there is often incomplete inactivation of pathogenic microorganisms, especially in the presence of organic matter (Gil et al., 2009; Luo, 2007). Moreover, there is a potential concern of toxicity due to compounds resulting from the reaction of chlorine with organic matter (Donato et al., 2010; Richardson et al., 1998; Zhang et al., 1996). These limitations have resulted in the search for alternative antimicrobials for reducing pathogens on fresh produce.

The goal of this dissertation was to examine the effect of natural, food-grade antimicrobials on the survival of foodborne pathogens on fresh produce. Specifically, the objectives were to (1) investigate the efficacy of four plant-derived compounds, namely carvacrol (CAR), *trans*-cinnamaldehyde (TC), eugenol (EUG) and β -resorcylic acid (BR) as a wash treatment in reducing *Salmonella* spp. on tomatoes, and (2) determine the efficacy of TC and CR in inactivating *E. coli* O157:H7 and *L. monocytogenes* on iceberg lettuce in the presence and absence of 10% organic matter.

References

- Donato, F., Zani, C., 2010. Chronic exposure to organochlorine compounds and health effects in adults: cancer, non-Hodgkin lymphoma. Review of literature. *Annali di Igiene: Medicina Preventiva e di Comunita* 22, 357-367.
- Gil, M. I., Selma, M. V., López-Gálvez, F., Allende, A., 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International journal of food microbiology* 134, 37-45.
- Luo, Y., 2007. Fresh-cut produce wash water reuse affects water quality and packaged product quality and microbial growth in romaine lettuce. *HortScience* 42, 1413–1419.
- Olaimat, A. N., Holley, R. A., 2012. Factors influencing the microbial safety of fresh produce: a review. *Food microbiology* 32, 1-19.
- Richardson, S. D., Thurston, Jr A.D., Caughran, T.V., Collette, T.W., Patterson, K.S., Lykins, Jr. B.W., 1998. Chemical by products of chlorine and alternative disinfectants. *Food Technology* 52, 58–61.
- Zhang, S., Farber, J.M., 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiology* 13, 311–321.

Chapter 2: Review of Literature

Current trends in fresh produce production and consumption.

The consumption of fresh produce in the United States has substantially increased in recent years (CAST, 2009; Singh et al., 2002; Hoelzer et al., 2012). This is largely due to significant advances in agronomic practices, processing, preservation, distribution and marketing of fresh produce. In addition, the awareness that fresh, whole, and minimally processed fruits, vegetables and juices have tremendous health benefits has also influenced consumers' food choices (Singh et al., 2002). Between 1970 and 2008, the per capita consumption of fresh fruits increased by 19 percent, and the consumption of vegetables (including potatoes) by 67 percent (CARD, 2004; CAST, 2009). In 1980, the per capita consumption of fresh vegetables was around 107.9 lbs per year, which increased to 180.5 lbs in 2008. Likewise, fresh fruit consumption increased from approximately 84.2 lbs in 1980 to 100.2 lbs in 2008 (USDA, 2008). Moreover, in the last three decades, an increase in fresh produce consumption has also led to a concomitant rise in the import of fresh fruit and vegetables by 155 and 265 percent, respectively. (CARD, 2004).

Among the various fresh produce items consumed in the U.S., lettuce and tomatoes are the most widely consumed (USDA ERS, 2013). The United States is the second largest lettuce producing country after China (AGMRC, 2009a). The U.S. production of lettuce in 2008 totaled approximately 91 million lbs (AGMRC, 2009a), 98% of which was cultivated domestically (AGMRC, 2009a). The monetary value of the U.S. lettuce industry in 2008 was approximately \$1.98 billion, making it the leading vegetable crop in terms of economic gains (AGMRC, 2009a). Moreover, the consumption of fresh market romaine and leaf lettuce increased from 10.6 lbs in 2005 to

15.1 lbs in 2008 (Tables for Fresh Vegetables, 2009). The United States remains a leading lettuce exporter, second behind Spain, accounting for 20 percent of dollar value of global exports (AGMRC, 2009a).

Tomatoes are a popular and commonly consumed fruit in the United States. By 2008, nearly 2 million tons of commercial fresh-market tomatoes were produced in the United States annually (AGMRC, 2009b). The per capita consumption of fresh tomatoes has also been increasing in recent years. For example, the annual per capita tomato consumption of 12.3 lbs in 1981 increased to 20.3 pounds in 2007 (AGMRC, 2009b). This is partly attributed to the increasing popularity of fresh-market tomato in salads and sandwiches, improved varieties, and a growing population of immigrants with preferences for vegetable diets (ERS, 2008). According to the National Agriculture Statistics Service (NASS), U.S. fresh-market tomato production in 2010 was valued at \$1.4 billion, the highest ranked fresh-market vegetable in the country (AGMRC, 2005).

Foodborne illness burden in the United States

Despite significant advancement in food safety and processing technology, foodborne illnesses remain a significant concern in the United States. Annually, an estimated 48 million cases of foodborne illness occur in the country, with approximately 128,000 hospitalizations, and 3,000 deaths (CDC, 2011). Foodborne illnesses cost an estimated \$ 78 billion each year in health-related expenses (Scharff, 2012). Some of the major pathogens associated with foodborne illnesses in the United States include non-typhoidal *Salmonella spp.*, *Campylobacter spp.*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Shigella spp.* and Norovirus (CDC, 1997a & 1997b; CDC, 2011; Davis et al., 1988; Farber et al., 1990).

Contamination of fresh produce

A major concern with increased fresh produce consumption is the potential risk of acquiring foodborne diseases. Fresh fruits and vegetables are considered to be potent sources of foodborne disease outbreaks in many parts of the world (Lynch et al., 2009). Contamination of fresh produce can occur at many points between the farm and the consumer (Gorny, 2006), including the use of infected seed, during production, harvesting, post-harvest handling, transport, distribution, storage, and food preparation. In the United States alone, the proportion of outbreaks linked to fresh produce increased from < 1% of all reported outbreaks with a known food vehicle in the 1970s to 6% in the 1990s (Sivapalasingam et al., 2004). According to the Center for Science in the Public Interest database of foodborne illness, 28,315 of 138,622 cases (20.4%) in the United States from 1990 through 2003 were associated with the consumption of fresh produce (Dewaai et al., 2006). At present, approximately 46% of all foodborne illnesses are caused by contaminated produce (CDC, 2013). The pathogens of concern reported with these foodborne illness outbreaks were *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Clostridium botulinum*, *Cryptosporidium* spp., *Cyclospora* spp., Hepatitis A virus, and Norwalk-like viruses (Sapers et al., 2006). The different types of fresh fruits and vegetables involved in the outbreaks include lettuce, alfalfa sprouts, bean sprouts, cilantro, parsley, scallions (green onions), tomatoes, radishes, and peppers (Beuchat, 1998). In the US, tomatoes, lettuce, and spinach are the major produce implicated in foodborne disease outbreaks (Zhuang et al., 1995; Sivapalasingam et al., 2004; CDC, 2007, 2008a; Gupta et al., 2007; Hanning et al., 2008; Wendel et al., 2009).

***Escherichia coli* O157:H7.**

Escherichia coli O157:H7 is a Gram negative, rod shaped, bacterium that causes enterohemorrhagic diarrhea in humans. The pathogen's virulence has been attributed to its ability to produce "Shiga-like" toxins or verotoxins, which can cause hemolytic uremic syndrome (HUS), which often results in kidney damage and/or failure. Of those who develop HUS, one-third is reported to have abnormal kidney function for many years, 3–5% reportedly die, a few require long-term dialysis, and another 8% develop other lifelong complications, such as high blood pressure, seizures, blindness, paralysis and procedure-related side-effects if surgery is required to remove a part of the bowel. Some of the common sources of *E. coli* O157:H7 include undercooked and/or contaminated ground beef, under-pasteurized milk, contaminated water and raw vegetables (Law, 2001). The Centers for Disease Control and Prevention (CDC) estimated that every year at least 2000 Americans are hospitalized, and about 60 die as a direct result of *E. coli* O157:H7 infection and its complications (Scallan et al., 2011).

Listeria monocytogenes.

Listeria monocytogenes is a Gram positive, motile, rod shaped, facultative anaerobic, psychrotrophic bacterium. *L. monocytogenes* is most commonly associated with contaminated raw milk, soft cheeses, raw meat, fish, poultry, ready-to-eat meat products and vegetables. The ability to be transmitted through ready-to-eat processed meats and dairy foods has made *L. monocytogenes* an important pathogen of significant public health concern (EFSA, 2007). *L. monocytogenes* can adapt to a wide range of food processing and storage conditions, including refrigeration temperatures and acidic or high salt environments (Nobmann et al., 2009). It has one of the highest case fatality

rates of all the foodborne infections, with a 20-30% mortality rate in susceptible individuals (De Valk et al., 2005). According to Scallan et al. (2011), an estimated 1600 foodborne listeriosis cases occur per year in the US, after adjusting for under-reporting. The health related costs due to *L. monocytogenes* is estimated at \$8.8 billion US dollars annually (Scharff, 2012). The population groups most commonly affected by foodborne listeriosis are pregnant women, neonates, the elderly, and people with a compromised immune system (Gerba et al., 1996). The condition may last from a few days to several weeks (Rocourt and Cossart, 1998). Mild cases of listeriosis are most often characterized by fever, severe headache, and vomiting (influenza-type symptoms). *L. monocytogenes* may result in abortion and intrauterine transmission of the pathogen to the fetuses and/or newborns, either before or during delivery (Ivanek et al., 2005). Severe cases of listeriosis often result in septicemia and/or meningoencephalitis. Listeriosis may also result in delirium and/or coma, and stillbirth. Additionally, surviving infants are usually impaired with developmental complications (Ivanek et al., 2005).

Salmonella spp.

Salmonella is a Gram negative, rod shaped, non-spore forming, motile, and facultative anaerobic bacterium. They are chemo-organotrophs, obtaining energy from oxidation and reduction reactions using organic sources. The bacterium is very effective in causing illness because of the type III secretion system, the AvrA toxin and its ability to suppress inflammation and immune response in intestinal epithelial cells (Liao et al., 2008). In addition, *Salmonella* can survive for several weeks in dry environments, and several months in water. Aquatic vertebrates, birds, reptiles, poultry, cattle, and sheep are frequently implicated as agents of *Salmonella* contamination. The pathogen can be found

in foods such as meat, raw egg and fresh produce. It is one of the most frequent causes of foodborne illness worldwide (WHO, 2013). In the United States, it is estimated that *Salmonella* causes 1.4 million cases of illness, approximately 20,000 hospitalizations and more than 500 deaths annually (Scallan et al., 2011). The total cost associated with *Salmonella* is estimated at \$14.6 billion annually in the US (Scharff, 2010). The major produce-borne outbreaks in the United States for last three decades are summarized in table 1.

Table 1. Outbreaks linked to fresh produce

Year	Fresh produce implicated	Source and affected regions	Causative organism	Cases	Reference
2013	Ready-to-eat salads	Trader Joe's grocery store	<i>E. coli</i> O157:H7	33	CDC, 2013
2013	Cucumbers	Daniel Cardenas Izabal and Miracle Green house Mexico 18 states affected	<i>Salmonella</i> Saintpaul	84	CDC, 2013
2012	Organic Spinach and Spring Mix Blend	State Garden of Chelsea, MA, 5 States affected	<i>E. coli</i> O157:H7	33	CDC, 2012
2011	Whole Cantaloupe	Jensen Farms, Colorado	<i>L. monocytogenes</i>	147	CDC, 2012

		28 States affected			
	Alfalfa sprouts and spicy sprouts	Evergreen Fresh Sprouts, LLC, 5 States affected	<i>S. Enteriditis</i>	25	CDC, 2012
	Romaine lettuce	10 States affectd	<i>E. coli</i> O157:H7	60	CDC, 2012
2010	Tiny Greens Alfalfa Sprouts or Spicy Sprouts	Jimmy John's restaurant outlets, 26 States and the District of Columbia affected	<i>Salmonella spp.</i>	140	CDC, 2012
	Alfalfa sprouts	J.H. Caldwell and Sons Inc. of Maywood, CA, 11 States affected	<i>S. Newport</i>	44	CDC, 2012
	Romaine lettuce	Subway in Illinois, 14 counties affected	<i>S. Hwittingfoss</i>	34	Falkenstein, 2010
	Shredded romaine lettuce	Freshway Foods, Sidney, Ohio. Michigan, and New York were also affected	<i>E. coli</i> O157: H7	19	FDA, 2010
2009	Alfalfa sprouts	6 states	<i>S. Saintpaul</i>	31	CDC, 2009
2008	Tomatoes	43 states affected	<i>S. enterica</i>	1329	CDC, 2008

2006	Uncooked Spinach	26 states affected	<i>E. coli</i> O157: H7	199	FDA, 2006
	Contaminated Lettuce (“Taco Bell and Taco John’s Outbreak”)	5 States affected	<i>E. coli</i> O157: H7	71	CDC, 2006
	Coleslaw	Massachusetts	<i>L. monocytogenes</i>	NA	FDA, 2006
	Tomato	21 States affected	<i>S. Typhimurium</i>	190	CDC, 2007
	Tomato	19 States affected	<i>S. Newport</i>	115	CDC, 2007
2005	Diced Tomatoes	3 States affected	<i>S. Braenderup</i>	76	CDC, 2007
	Diced Roma Tomatoes	Theme Park In Florida	<i>S. Javiana</i>	141	Srikantiah et al., 2005
2002	Tomato	22 States affected	<i>S. Newport</i>	552	CDC, 2007
1998	Tomato	8 States affected	<i>S. Baildon</i>	81	Cummings et al., 2001
1996	Mesclun Lettuce	Connecticut, Illinois	<i>E. coli</i> O157:H7	NA	Hilborn, 1999
1995	Lettuce	Montana	<i>E. coli</i> O157:H7	40	Ackers, 1998
1993	Tomato	4 States affected	<i>S. Montevideo</i>	100	Hedberg et al., 1999
1990	Packing House	4 States affected	<i>S. Javiana</i>	176	Hedberg et

	in South Carolina				al., 1999
1981	Cabbage	Nova Scotia, Canada	<i>Listeria spp.</i>	41	Schlech et al., 1983
1979	Raw Celery, Tomatoes and Lettuce	Boston, MA	<i>L. monocytogenes</i>	23	Ho et al., 1986

Methods for reducing pathogens on fresh produce

Some of the common decontamination methods used for fruits and vegetables in the industry are waxing, spraying, washing and dipping in detergent solutions (Beier *et al.* 2004). These methods are commonly done in combination with various food grade acids and sanitizers. The recommended treatments used in the industry consist of using chlorine (chlorine gas, sodium hypochlorite, calcium hypochlorite and chlorine dioxide), bromine, iodine, hydrogen peroxide, tri-sodium phosphate, quaternary ammonium compounds, ozone, ionizing irradiation, acids, all organic acids including lactic and citric acids (Beuchat, 1998).

Chlorine

Washing produce with sodium-chlorinated water (NaClO) is the most commonly used method to remove pathogens from fruit and vegetable surfaces (Gunduz et al., 2009). Chlorine wash systems (most often water flumes) are used by numerous (76%) fresh produce manufacturers for decontamination (Gunduz et al., 2009; Zhang et al., 2009). Water containing 50–200 ppm of chlorine is widely used in processing plants for

washing fresh produce received from farms (Gunduz et al., 2009). Although chlorine is used to wash fresh produce, including tomatoes, the fruit washed with chlorine (40 – 60 ppm) was implicated as a source of food-borne disease in outbreaks (Wei et al., 1995). Zhuang et al. (1995) reported that tomatoes washed (2 minutes) with chlorine at 110 ppm were not significantly different from those washed with 320 ppm in their *Salmonella* Montevideo populations. They reported that there was incomplete inactivation even when chlorine was used at high concentrations (320 ppm).

Delaquis et al. (2002) inoculated cut iceberg lettuce with *E. coli* O157:H7 and *L. monocytogenes* before washing for 3 min in cold (4°C/39°F) (current method used in industry) and warm (47°C/117°F) water containing 100 ppm total chlorine and enumerated surviving populations of the bacteria. They found that after storage for up to 14 days, *E. coli* O157:H7 and *L. monocytogenes* populations declined when washed with cold water containing chlorine, and the pathogen counts were greater after washing with warm water with chlorine. Li et al. (2001) also studied the survival and growth of *E. coli* O157:H7 on lettuce treated with 20 ppm chlorine at either 20°C or 50°C, and then storing at 5°C for 18 days or at 15°C for 7 days. They concluded that populations declined throughout storage at 5°C, but increased by 2.3 to 3.2 log CFU/g within 2 days at 15°C. Further, the counts increased at a lower rate through 7 days of storage at that temperature.

Toxicity of chlorine compounds

Active hypochlorite is believed to lose its efficacy by reacting with nitrogen-containing compounds in foods, resulting in the formation of halogenated organic compounds (Wei et al., 1985). Fawell (2000) reported that chlorine could react with naturally occurring organic matter to form chloroform, and other trihalomethanes such as

bromodichloromethane, chlorodibromomethane and bromoform. These compounds, especially trihalomethanes, have been shown to increase tumors of the liver, kidney or large intestine in rats and mice (Fawell, 2000) and are toxic to humans (Chen et al., 2003).

In light of the aforementioned limitations of chlorine, several non-chlorine interventions have been investigated to reduce bacterial populations on fresh produce. Sanitizing alternatives such as electrolyzed water (Bari et al., 2003; Deza et al., 2003; Park et al., 2008), ozonated water (Chaidez et al., 2007) and calcinated calcium (Bari et al., 2002) have been evaluated for their effectiveness in killing pathogens such as *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on tomatoes and other fresh produce.

Peroxyacetic acid

Peroxyacetic acid (PA) is a strong oxidizing agent, non-corrosive and is often added to produce wash water at a maximum concentration of 80 ppm (Bhagat, 2010). In a study by Park et al. (1999), PA significantly reduced *Salmonella* and *E. coli* O157:H7 on cantaloupe and honeydew melon. However, in comparison to solutions of sodium hypochlorite, chlorine dioxide and ozone, PA was shown to be the least effective in reducing bacterial load on fresh produce (Rodgers et al., 2004).

Hydrogen peroxide

Hydrogen peroxide is an oxidizing agent and its rapid breakdown into non-harmful components makes it a good sanitizing agent for food surfaces (Rico et al., 2007). It can have a negative effect on products such as shredded lettuce because of the adverse effect on organoleptic properties (Rico et al., 2007; Beauchat., 1998).

Ozone

The advantages of ozone for decontaminating fresh produce are its strong penetrability and decomposition into non-toxic products (Rodgers et al., 2004; Rico et al., 2007). Treatment with ozonated water increased shelf life of apples, grapes, oranges, pears, raspberries, and strawberries (Beuchat, 1998), however, some changes in fruit quality, especially on tomatoes occurred (Daş et al., 2006). Ozone is also corrosive and needs to be generated on demand, thereby making it difficult to transport (Beuchat, 1998).

Plant compounds

The use of natural antimicrobial molecules for inactivating pathogenic microorganisms has received renewed attention due to concerns for toxicity of synthetic chemicals (Abe et al., 1995; Salamci et al., 2007). Plants have served as the source of many drugs that contribute to human health and well-being. The active components in several herbal and traditional plant-derived medicines contain polyphenols that are believed to improve health. The antimicrobial properties of some of these plant-derived polyphenols have been demonstrated (Burt, 2004; Holley and Patel, 2005), and a variety of active components in these oils has been identified. *Trans*-cinnamaldehyde (TC) is an aromatic aldehyde present as a major component of bark extract of cinnamon (*Cinnamomum zeylandicum*) (Lens-Lisbonne et al., 1987). Carvacrol (CAR) is an antimicrobial ingredient in oregano oil obtained from *Origanum glandulosum* (Bandahou et al., 2008). Eugenol (EUG) is an active ingredient in the oil obtained from cloves (*Eugenia caryophyllis*; Ali et al., 2005). β -resorcylic acid (BR; 2, 4 dihydroxy benzoic acid) is a phytophenolic compound widely distributed among the angiosperms (Bolton et

al., 1986), and is a secondary metabolite that plays a key role in the biochemistry and physiology of plants (Friedman et al., 2003). All of these molecules are classified by the United States Food and Drug Administration as GRAS (generally recognized as safe) molecules. Previous research conducted with sumac, oregano (Gunduz et al., 2010) and myrtle oil (Gunduz et al., 2009) demonstrated their antimicrobial activity against *Salmonella* on tomatoes and lettuce. Sumac (4%), oregano (100 ppm) and myrtle oil (1000 ppm) decreased *Salmonella* counts by 2.38, 2.78 and 1.66 log respectively on lettuce after 20 min of washing. However, no rapid reductions with these molecules were observed.

Antimicrobial mechanisms of plant molecules

Plant-derived molecules contain a number of different chemical groups in their structure, and their antimicrobial activity is attributed to more than one specific mechanism (Skandamis et al., 2001; Carson et al., 2002; Burt, 2004). A critical property of these molecules is their hydrophobicity, which helps them to target the lipid-containing bacterial cell membrane and mitochondria (Knobloch et al., 1986; Sikkema et al., 1994). This makes these membranes more permeable, leading to leakage of ions and other cell contents (Cox et al., 2000; Carson et al., 2002; Ultee et al., 2002). Besides the effect on cell membranes, TC is also reported to kill bacteria by inhibiting energy generation and glucose uptake (Gill et al., 2004), and by their inhibitory effect on enzymes such as amino acid decarboxylases (Wendakoon et al., 1995). Because of the multiple antimicrobial mechanisms, the potential of bacteria for developing resistance to plant antimicrobials is believed to be smaller (Ohno et al., 2003; Domadia et al., 2007).

In light of the increasing role of fresh produce in disease outbreaks, and consumer demand for naturally treated foods, there is a need for safe and effective antimicrobial treatments that can rapidly reduce pathogenic populations on fresh produce. The current industry-employed method of chlorine wash is not effective, and may have adverse health effects. In addition, chlorine is not effective in inactivating pathogens in the presence of organic matter. The outbreaks of *Salmonella* on tomatoes, and *E. coli* O157: H7 on iceberg lettuce raises concerns over the existing decontamination methods for fresh produce. Therefore, the present study was undertaken with the goal of investigating the antimicrobial potential of plant-derived molecules for reducing *Salmonella enterica*, *E. coli* O157: H7 and *L. monocytogenes* on fresh produce.

The specific objectives include:

1. To investigate the efficacy of plant-derived compounds for inactivating *Salmonella* spp. on tomatoes.
2. To investigate the efficacy of plant-derived compounds for decreasing *E. coli* O157: H7 and *Listeria monocytogenes* populations on lettuce.

References

- Abe T., Crooked, L., Hill, C., 1995. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology* 28, 169 – 185.
- Ackers, M.L., Mahon, B.E., Leahy, E., Goode, B., Damrow, T., Hayes, P.S., Bibb, W.F., Rice, D.H., Barrett, T.J., Hutwagner, L., Griffin, P.M., Slutsker, L., 1998. An Outbreak of *Escherichia coli* O157:H7 Infections Associated with Leaf Lettuce Consumption. *Journal of Infectious Diseases* 177, 1588–1593.
- AGMRC., 2009a. Agriculture Marketing Resource Center. Lettuce profile. Accessed at: http://www.agmrc.org/commodities__products/vegetables/lettuce_profile.cfm Accessed 2 November 2009. Accessed on 05/11/2010.
- AGMRC., 2009b. Agriculture Resource Marketing Center. Fresh tomatoes profile. Accessed at: http://www.agmrc.org/commodities__products/vegetables/fresh_tomatoes_profile.cfm. Accessed on: 05/11/2010.
- AGMRC., 2005. Agriculture Resource Marketing Center. Fresh tomatoes profile. Accessed at: http://www.agmrc.org/commodities__products/vegetables/fresh_tomatoes_profile.cfm. Accessed on: 04/11/2014.
- Ali, S.M., Khan, A.A., Ahmed, I., Musaddiq, M., Ahmed, K.S., Polasa, H., Rao, V.L., Habibullah, C.M., Sechi, L.A., Ahmed, N., 2005. Antimicrobial activities of eugenol and cinnamaldehyde against the human gastric pathogen *Helicobacter pylori*. *Annals of Clinical Microbiology and Antimicrobials* 4, 20–26.
- Alvarado-Casillas, S., Ibarra-Sánchez, S., Rodríguez-García, O., Martínez-González, N., Castillo, A., 2007. Comparison of rinsing and sanitizing procedures for

- reducing bacterial pathogens on fresh cantaloupes and bell peppers. *Journal of Food Protection* 70, 655– 660.
- Bari, M.L., Inatsu, Y., Kawasaki, S., Nazuka, E., Isshiki, K., 2002. Calcinated calcium killing of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on the surface of tomatoes. *Journal of Food Protection* 65, 1706–1711.
- Bari, M.L., Inatsu, Y., Kawasaki, S., Nazuka, E., Isshiki, K., 2003. Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on the surfaces of tomatoes. *Journal of Food Protection* 66, 542–548.
- Beier, R.C., Pillai, S.D., Phillips, T.D. and Ziprin, R. L. 2004. Preharvest and postharvest food safety: contemporary issues and future directions. 4:46-50.
- Bendahou M., Muselli, A., Grignon-Dubois, M., Benyoucef, M., Desjobert, J., Bernardini, A., Costa, J., 2008. Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. essential oil and extract obtained by microwave extraction: Comparison with hydrodistillation. *Food Chemistry* 106, 132–139.
- Beuchat, L.R. 1996. Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection* 59, 204-216.
- Beuchat, L.R., 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Report WHO/FSF/FOS/98.2. World Health Organization, Geneva.
- Bhagat, A. R., 2010. Modeling Critical Factors to Optimize the Treatment of Selected Fruits and Vegetables with Chlorine Dioxide Gas Using a Miniaturized Industrial-Size Tunnel System. West Lafayette, Indiana, Purdue University.

- Bolton, G.W., Nester, E.W., Gordon, M.P., 1986. Plant phenolic compounds induce expression of the *Agrobacterium tumefaciens* loci needed for virulence. *Science* 232, 983-985.
- Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in food – a review. *International Journal of Food Microbiology* 94, 223–253.
- California tomato commission. Accessed at: <http://www.tomato.org/Heirloom-Tomato.html>. Accessed on: 05/11/2010.
- CARD., 2004. Center for Agricultural and Rural Development. Risk free Farming? Iowa Ag Review. 10, 1-12. Accessed at: http://www.card.iastate.edu/iowaag_review/winter04/article4.aspx. Accessed on 05/28/2010.
- Carson, C.F., Mee, B.J., Riley, T. V., 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrobial Agents and Chemotherapy* 46, 1914-1920.
- CBC., 2004. Indepth: Inside Walkerton. Canada's worst-ever *E. coli* contamination CBC News Online. Updated Dec. 20, 2004. Accessed at: <http://www.cbc.ca/news/background/walkerton/>. Accessed on 05/12/2010.
- CDC., 1997a. Centers for Disease Control and Prevention. Update: Outbreaks of Cyclosporiasis. *Morbidity and Mortality Weekly Report* 46, 521-523.
- CDC., 1997b. Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts – Michigan and Virginia. June – July 1997. *Morbidity and Mortality Weekly Report* 46, 741-744.

- CDC., 2002. Centers for Disease Control and Prevention. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupe from Mexico. United States and Canada, 2000–2002. Morbidity and Mortality Weekly Report 51, 1044–1047.
- CDC., 2005. Center for Disease Control and Prevention. Frequently Asked Questions. January 10, 1-13.
- CDC., 2006. Centers for Disease Control and Prevention. E. coli Outbreak - Update: Dec. 14, 2006 CDC Foodborne and Diarrheal Diseases Branch.
- CDC., 2007. Centers for Disease Control and Prevention. Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants. United States, 2005–2006. Morbidity and Mortality Weekly Report 56, 909–911.
- CDC., 2008a. Centers for Disease Control and Prevention. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items. United States, 2008. Morbidity and Mortality Weekly Report 57, 929–934.
- CDC., 2008b. Centers for Disease Control and Prevention. Interpretation of Epidemic Curves During an Active Outbreak "FDA Warning on Serious Foodborne E.coli O157:H7 Outbreak".
- CDC., 2009. Centers for Disease Control and Prevention. *Listeria* outbreak linked to alfalfa sprouts. Accessed at: <http://foodpoisoning.pritzkerlaw.com/archives/listeria-cdc-listeria-outbreak-linked-to-alfalfa-sprouts.html>. Accessed on 05/11/2010.

- CDC., 2011. Center for Disease Control and Prevention. Estimates of Foodborne Illness in the United States. Accessed at: <http://www.cdc.gov/foodborneburden/>. Accessed on: 09/21/12.
- CDC., 2011. Estimates of Foodborne illness in the United States. Accessed at: <http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html#illness>.
- CDC., 2011. Multistate Outbreak of Listeriosis Linked to Whole Cantaloupes from Jensen Farms, Colorado. Accessed at: <http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html>. Accessed on: 03/11/13.
- CDC., 2012. Center for Disease Control and Prevention. Multistate Foodborne Outbreak Investigations. Accessed at: <http://www.cdc.gov/outbreaknet/outbreaks.html>. Accessed on: 12/12/12.
- CDC., 2013. Center for Disease Control and Prevention. Estimates of Foodborne illness in the United States. Available at: <http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html#illness>.
- Chaidez, C., Lopez, J., Vidales, J., Campo, N.C., 2007. Efficacy of chlorinated and ozonated water in reducing *Salmonella* typhimurium attached to tomato surfaces. International Journal of Environmental Health Research 17, 311–318.
- Chen, J., Douglas, G. C., Thirkill, T. L., Lohstroh, P. N., Bielmeier, N. R., Narotsky, M. G., Best, D. S., Harrison, R. A., Natarajan, K., Pegram, R. A., Overstreet, J. W., Lasley, B. L., 2003. Effect of Bromodichloromethane on Chorionic Gonadotrophin Secretion by Human Placental Trophoblast Cultures. Toxicological Sciences, 76, 75– 82.

- Cox, N.A., Richardson, A.L.J., Buhr, A.D.R.J., Fedorka-Cray, A.P.J., Bailey, B.J.S., Wilson, B.J.L., Hiett, K.L., 2006. Natural presence of *Campylobacter* spp. in various internal organs of commercial broiler breeder hens. *Avian Diseases* 50, 450–453.
- Cummings, K., Barrett, E., Mohle-Boetani, J.C., Brooks, J.T., Farrar, J., Hunt, T., Fiore, A., Komatsu, K., Benson Werner, S., Slutsker., L., 2001. A Multistate Outbreak of *Salmonella enterica* Serotype Baildon Associated with Domestic Raw Tomatoes. *Emerging Infectious Diseases* 7, 1046-1048.
- Daş, E., Gürakan, G. C., Bayındırlı, A., 2006. "Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella Enteritidis* on cherry tomatoes." *Food Microbiology* 23(5), 430-438.
- Davis, H., Taylor, J.P., Perdue, J.N., Stelma, Jr, G.N., Humphrey, Jr., J.M., Rowntree, R. III, Greene, K.D., 1988. A shigellosis outbreak traced to commercially distributed shredded lettuce. *American Journal of Epidemiology* 128, 1312-1321.
- Delaquis, P.J., Stewart, S., Toivonen, P.M.A., Moyls, A.L., 1999. Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. *Food Research International* 32, 7–14.
- Dewaal, C.S., Hicks, G., Barlow, K., Alderton, L., Vegosen, L., 2006. Foods associated with food-borne illness outbreaks from 1990 through 2003. *Food Protection Trends* 26, 466–473.
- Deza, M.A., Araujo, M., Garrido, M.J., 2003. Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water. *Letters in Applied Microbiology* 37, 482–487.

- Domadia, P., Swarup, S., Bhunia, A., Sivaraman, J., Dasgupta, D., 2007. Inhibition of bacterial cell division protein FtsZ by cinnamaldehyde. *Biochemical Pharmacology* 832, 831-840.
- Doyle, M P., 2000. Reducing foodborne disease. *Food Technology* 54, 130.
- ERS., USDA., 2008. Accessed at: <http://www.ers.usda.gov/data/foodconsumption/app/availability.aspx>. Accessed on 05/11/2010.
- ERS., USDA., 2009. Tables for Fresh Vegetables.
- ERS., USDA., Foodborne illness calculator. Accessed at: <http://www.ers.usda.gov/data/foodborneillness/>. Accessed on 05/12/2010.
- ERS., USDA., Vegetable and Melons: Tomatoes. Accessed at: <http://www.ers.usda.gov/Briefing/Vegetables/tomatoes.htm>. Accessed on 05/11/2010.
- Falkenstein, D., 2010. Salmonella Hvittingfoss: a rare strain in the Illinois Subway outbreak. *Food Poison Journal*. Accessed at: <http://www.foodpoisonjournal.com/2010/06/articles/foodborne-illness-outbreaks/salmonella-hvittingfoss-a-rare-strain-in-the-illinois-subway-outbreak/>. Accessed on: 06/07/2010.
- Farber, J.M., Carter, A.D., Varughese, P.V., Ashton, F.E. Ewan, E.P., 1990. Listeriosis traced to the consumption of alfalfa tablets and soft cheese. *New England Journal of Medicine* 332, 338.
- Fawell, J., 2000. Risk assessment case study – chloroform and related substances. *Food and Chemical Toxicology*, 38, 91-95.
- FDA., 2006. U.S. Food and Drug Administration. Accessed at: <http://www.fda.gov/bbs/topics/NEWS/2006/NEW01450.html>. Accessed on 10/20/2009.

- FDA., 2006. U.S. Food and Drug Administration. Boston Salads & Provisions Co., Inc. Recalls Cole Slaw Because of Possible Health Risk., Safety, Recall-Firm Press Release. Accessed at: <http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2006/ucm112061.htm>. Accessed on 05/11/2010.
- FDA., 2010. U.S. Food and Drug Administration. FDA news release: FDA Supports Freshway Foods Voluntary Recall of Lettuce. Accessed at: <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm211145.htm>. Accessed on 05/11/2010.
- FHO/WHO., 2005. Drug resistant *Salmonella*. World Health Organization. Accessed at: <http://www.who.int/mediacentre/factsheets/fs139/en/>. Accessed on 05/12/2010.
- Frenzen, P.D., Drake, A., Angulo, F.J., The emerging infections program foodnet working group., 2005. Economic Cost of Illness Due to *Escherichia coli* O157 Infections in the United States. *Journal of Food Protection* 68, 2623–2630.
- Friedman M., Henika, P.R., Mandrell, R.E., 2003. Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection* 66, 1811-1821.
- Gerba, C.P., Rose, J.B., Haas, C.N., 1996. Sensitive populations: Who is at the greatest risk? *International Journal of Food Microbiology* 30, 113–123.
- Gill, A.O., Holley, R.A., 2004. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *International Journal of Food Microbiology* 108, 1-9.

- Gonzalez, R.J., Luo, Y., Ruiz-Cruz, S., McEvoy, J.L., 2004. Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *Journal of Food Protection* 67, 2375–2380.
- Gorny, J.R., 2006. Microbial contamination of fruits and vegetables, p.3-32. *In* G. M. Sapers, J. R. Gorny, and A. E. Yousef (ed.), *Microbiology of fruits and vegetables* CRC press, Boca Raton, FL.
- Gupta, S.K., Nalluswami, K., Snider, C., Perch, M., Balasegaram, M., Burmeister, D., Lockett, J., Sandt, C., Hoekstrah, R.M., Montgomery, S., 2007. Outbreak of *Salmonella* Braenderup infections associated with Roma tomatoes, northeastern United States, 2004: a useful method for subtyping exposures in field investigations. *Epidemiology and Infection* 135, 1165–1173.
- Hanning, I.R., Nutt, J.D., Ricke, S.C., 2009. Salmonellosis Outbreaks in the United States Due to Fresh Produce: Sources and Potential Intervention Measures. *Foodborne Pathogens and Disease* 6, 635-648.
- Hedberg, C.W., Angulo, F.J., White, K.E., Langkop, C.W., Schell, W.L., Stobierski, M.G., Schuchat, A., Besser, J.M., Dietrich, S., Helsel, L., Griffin, P.M., McFarland, J.W., Osterholm, M.T., 1999. Outbreaks of salmonellosis associated with eating uncooked tomatoes: implications for public health *Epidemiology and Infection* 122, 385–393.
- Hilborn, E.D. Mermin, J.H., Mshar, P.A., Hadler, J.L., Voetsch, A., Wojtkunski, C., Swartz, M., Mshar, R., Lambert-Fair, M.A., Farrar, J.A., Glynn, M.K., Slutsker, L., 1999. A Multistate Outbreak of *Escherichia coli* O157:H7 Infections

- Associated With Consumption of Mesclun Lettuce. Archives of Internal Medicine 159, 1758-1764.
- Ho, J.L., Shades, K.N., Friedland, G., Eckind, P., Fraser, D.W., 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Archives of International Medicine 146, 520–524.
- Hoelzer, K., Pouillot, R., Egan, K., Dennis, S., 2012. Produce Consumption in the United States: An Analysis of Consumption Frequencies, Serving Sizes, Processing Forms, and High-Consuming Population Subgroups for Microbial Risk Assessments. Journal of Food Protection, 75, 328-340.
- Holley, R.A., Patel, D., 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. International Journal of Food Microbiology 22, 273–292.
- Inatsu, Y., Bari, M.L., Kawasaki, S., Isshiki, K., Kawamoto, S., 2005. Efficacy of acidified sodium chlorite treatments in reducing *Escherichia coli* O157:H7 on Chinese cabbage. Journal of Food Protection 68, 251–255.
- Ivanek, R., Gröhn, Y.T., Tauer, T.W., Wiedmann, W., 2005. The Cost and Benefit of *Listeria Monocytogenes*. Food Safety Measures 45, 514-523.
- Keskinen, L.A., Burke, A., Annous, B.A., 2009. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157:H7 from lettuce leaves International Journal of Food Microbiology 132, 134–140.
- Knobloch, K., Weigand, H., Weis, N., Schwarm, H.M., Vigneschow, H., 1986. Action of terpenoids on energy metabolism. In: Brunke, E.J. (Ed.), Progress in Essential Oil

- Research: 16th International Symposium on Essential Oils. De Gruyter, Berlin, pp. 429– 445.
- Law, D. 2000. Virulence factors of *Escherichia coli* O157 and other Shiga toxin producing *E. coli*. *Journal of Applied Microbiology*, 88, 729-745.
- Lens-Lisbonne C., Cremieux, A., Maillard, C., Balansard, B., 1987. Methodes d'evaluation de l'activite antibacterienne des huiles essentielles : application aux essences de thym et de cannelle. *Journal de pharmacie de Belgique* 42 : 297 – 302. In: Burt S. *Essential oils: their antibacterial properties and potential applications in food – a review*. *International Journal of Food Microbiology* 2004; 94, 223–253.
- Li, Y., Brackett, R.E., Chen, J., Beuchat, L.R., 2001. Survival and growth of *Escherichia coli* O157:H7 inoculated onto cut lettuce before or after heating in chlorinated water, followed by storage at 5 or 15°C. *Journal of Food Protection* 64, 305–309.
- Liao, A. P., Petrof, E. O., Kuppireddi, S., Zhao, Y., Xia, Y., Claud, E. C., Sun, J. 2008. *Salmonella* type III effector AvrA stabilizes cell tight junctions to inhibit inflammation in intestinal epithelial cells. *PLoS One*, 3(6), e2369.
- Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases* 5, 607–625.
- NASS., National Agriculture Statistical Service. Accessed at: <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1177>. Accessed on 05/11/2010.

- Nei, D., Choi, J.W., Bari, M.L., Kawasaki, S., Kawamoto, S., Inatsu, Y., 2009. Efficacy of chlorine and acidified sodium chlorite on microbial population and quality changes of spinach leaves. *Foodborne Pathogen and Disease* 6, 541–546.
- Nobmann, P., Smith, A., Dunne, J., Henahan, G., Bourke, P., 2009. The antimicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against *Listeria* spp. and food spoilage microorganisms. *International Journal of Food Microbiology* 128, 440-445.
- Ohno, T., Kita, M., Yamaoka, Y., Imamura, S., Yamamoto, T., Mitsufuji, S., Kodama, T., Kashima, K., Imanishi, J., 2003. Antimicrobial activity of essential oils against *Helicobacter pylori*. *Helicobacter* 8, 207-215.
- Pao, S., Kelsey, D.F., Khalid, M.F., Ettinger, M.R., 2007. Using aqueous chlorine dioxide to prevent contamination of tomatoes with *Salmonella enterica* and *Erwinia carotovora* during fruit washing. *Journal of Food Protection* 70, 629–634.
- Park, C. M., Beuchat, L. R., 1999. "Evaluation of Sanitizers for Killing *Escherichia coli* O157:H7, *Salmonella*, and Naturally Occuring Microorganisms on cantaloupes, Honeydew Melons, and Asparagus." *Dairy Food and Environmental Sanitation* 19: 842-847.
- Park, E.J., Alexander, E., Taylor, G.A., Costa, R., Kang, D.H., 2008. Fate of foodborne pathogens on green onions and tomatoes by electrolysed water. *Letters in Applied Microbiology* 46, 519–525.
- Rico, D., Martín-Diana, A. B., Barat, J. M., Barry-Ryan, C., 2007. "Extending and measuring the quality of fresh-cut fruit and vegetables: a review." *Trends in Food Science & Technology*, 18(7), 373-386.

- Rocourt, J., Cossart, P., 1998. *Listeria monocytogenes*. In: Food Microbiology Fundamentals and Frontiers. pp. 337–352. Doyle, M.P., Beuchat, L.R., and Montville, T.J. eds. Washington D.C.: ASM Press.
- Rodgers, S. L., Cash, J. N., Siddiq, M. and Ryser, E. T. (2004). "A Comparison of Different Chemical Sanitizers for Inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in Solution and on Apples, Lettuce, Strawberries, and Cantaloupe." Journal of Food Protection 67(4), 721-731.
- Salamci, E., Kordali, S., Kotan, R., Cakir, A., Kaya, Y., 2007. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum*. Biochemical Systematics and Ecology 35, 569-581.
- Sapers, G.M., Jones, D.M., 2006. Improved Sanitizing Treatments for Fresh Tomatoes. Journal of Food Science 71, 252-256.
- Scharff, R.L., 2010. Health Related Costs from foodborne illness in the United States. Produce safety project at Georgetown University. Accessed at: <http://www.producesafetyproject.org/admin/assets/files/Health-Related-Foodborne-Illness-Costs-Report.pdf-1.pdf>. Accessed on 05/13/2010.
- Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S., Broome, C.V., 1983. Epidemic listeriosis – evidence for transmission by food. New England Journal of Medicine 308, 203–206.
- Sikkema, J., De Bont, J.A.M., Poolman, B., 1994. Interactions of cyclic hydrocarbons with biological membranes. The Journal of Biological Chemistry 269, 8022-8028.

- Singh, N., Singh, R.K., Bhunia, A.K., Stroshine, R.L., 2002. Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* O157:H7 on lettuce. *Lebensmittel-Wissenschaft und-Technologie* 35, 720-729.
- Sivapalasingam, S., Friedman, C.R., Cohen, L., Tauxe, R.V., 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection* 67, 2342–2353.
- Skandamis, P.N., Koutsoumanis, K., Fasseas, K., Nychas, G.J.E., 2001. Inhibition of oregano oil and EDTA on *Escherichia coli* O157: H7. *Italian Journal of Food Science* 13, 65-75.
- Srikantiah, P., Bodager, D., Toth, B., Kass-Hout, T., Hammond, R., Stenzel, S., Hoekstra, R.M., Adams, J., Van Duyne, S., Mead, P.S., 2005. Web-based Investigation of Multistate Salmonellosis Outbreak. *Emerging Infectious Diseases* 11, 4.
Accessed at: <http://www.cdc.gov/ncidod/eId/vol11no04/04-0997.htm>. Accessed on 05/13/2010.
- Stopforth, J.D., Mai, T., Kottapalli, B., Samadpour, M., 2008. Effect of acidified sodium chlorite, chlorine, and acidic electrolyzed water on *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated onto leafy greens. *Journal of Food Protection* 71, 625– 628.
- Ultee, A., Kets, E.P.W., Smid, E.J., 1999. Mechanisms of action of carvacrol on the food-borne-pathogen *Bacillus cereus*. *Applied Environmental Microbiology* 65, 4606-4610.

- USDA ERS, 2013. Ag and Food statistics: Charting the essentials. Available at <http://www.ers.usda.gov/data-products/ag-and-food-statistics-charting-the-essentials.aspx#.U0gF1F4t1LY>.
- Vela´zquez, L.C., Barbinia, N.B., Escuderoa, M.E., Estradaa, C.L., Guzma´n, A.M.S., 2008. Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157:H7 and *Yersinia enterocolitica* on fresh vegetables. *Food Control* 20, 262–268.
- Wei, C.I., Cook, D.L., Krik, J.R., 1985. Use of chlorine compounds in the food industry. *Food Technology* 39, 107–115.
- Wei, C.I., Huang, T.S., Kim, J.M., Lin, W.F., Tamplin, M.L., Bartz, J.A., 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. *Journal of Food Protection* 58, 829–836.
- Weisburger, J. H. 1999. Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. *Food and chemical toxicology*, 37, 943-948.
- Wendakoon, C.N. Sakaguchi, M., 1995. Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. *Journal of Food Protection* 56, 410-413.
- Wendel AM, Johnson DH, Sharapov U, Grant J, Archer JR, Monson T, Koschmann C, and Davis JP. 2009. Multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of packaged spinach, August–September 2006: the Wisconsin investigation. *Clin. Infect. Dis.* 48:1079–1086.
- WHO, 2013. *Salmonella* (non-typhoidal) Fact sheet. <http://www.who.int/mediacentre/factsheets/fs139/en/>

Zhuang, R.Y., Beuchat, L.R., Angulo, F.J., 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied Environmental Microbiology* 61, 2127–2131.

Chapter 3: Inactivation of *Salmonella* spp. on tomatoes by plant molecules

(Published in International Journal of Food Microbiology, 2010, 44, 464-8)

ABSTRACT

The efficacy of carvacrol (CAR), *trans*-cinnamaldehyde (TC), eugenol (EUG) and β -resorcylic acid (BR) as a wash treatment for reducing *Salmonella* spp. on tomatoes was investigated. Plum tomatoes inoculated with a six-serotype mixture of *Salmonella* (10^8 CFU) were subjected to washing in sterile deionized water (control) or deionized water containing chlorine (100 ppm), CAR (0.25 and 0.75%), TC (0.5 and 0.75%), EUG (0.25 and 0.75%), or BR (0.75 and 1.0%) for 15 sec, 1min, and 3 min. The plant molecules were more effective ($P < 0.05$) in reducing *Salmonella* on tomatoes compared to washing in water and chlorine. Both concentrations of CAR and TC, and 0.75% EUG decreased *Salmonella* counts on tomatoes by ~ 6.0 log CFU/ml at 1 min. Both concentrations of BR decreased the pathogen on tomatoes to undetectable levels at 3 min of exposure. Washing of tomatoes in deionized water and chlorine for 3 min reduced *Salmonella* by ca. 2.0 and 4.0 log CFU/ml, respectively. No *Salmonella* was detected in the wash water containing the plant molecules or chlorine, whereas a substantial population of the pathogen survived in the control wash water (~ 4.0 log CFU/ml). Moreover, none of the dipping treatments had any effect on the red color of tomatoes ($P > 0.05$). Results indicate that CAR, TC, EUG and BR could be used effectively to kill *Salmonella* on tomatoes, but additional studies on sensory and quality characteristics of tomatoes treated with plant molecules are warranted.

Key words: *Salmonella*, tomatoes, carvacrol, *trans*-cinnamaldehyde, eugenol, β -resorcylic acid

1. Introduction

Fruits and vegetables constitute an important portion of the American diet. From 1980 to 2001, the per capita consumption of fresh fruits and vegetables in the United States increased by 19 and 29%, respectively (CARD, 2004). In the U.S. alone, the proportion of outbreaks linked to fresh produce increased from less than 1% of all reported outbreaks with a known food vehicle in the 1970s to 6% in the 1990s (Sivapalasingam et al., 2004). Among the foodborne pathogens in the U.S., *Salmonella* constitute one of the most prevalent bacteria, causing an estimated 1.6 million foodborne illnesses with an annual cost of ~ \$14 billion (Scharff, 2010). Although salmonellosis has been largely associated with the consumption of contaminated foods of animal origin, fresh tomatoes have been linked to major outbreaks of the pathogen time after time. Multi-state outbreaks involving *Salmonella* serotypes such as *S. Baildon*, *S. Braenderup*, *S. Javaina*, *S. Montevideo*, *S. Newport*, *S. Saintpaul* and *S. Typhimurium* associated with consumption of tomatoes have been reported. The largest *Salmonella* outbreak in the U.S., associated with *S. Saintpaul*, was linked to consumption of hot peppers and possibly tomatoes (CDC, 2008). Epidemiological investigations have revealed that tomatoes can potentially get contaminated with *Salmonella* from a variety of sources, including irrigation water, wash water, food preparation environments and animals (Hanning et al., 2009).

A wide range of chemical sanitizers and physical treatments have been investigated for killing *Salmonella* on tomatoes with varying degrees of success (Beuchat, 1998; Lang et al., 2004). Although chlorine is one of the common sanitizers used in the industry to decontaminate fresh produce, tomatoes washed with chlorine (40 –

60 ppm) were involved in outbreaks (Wei et al., 1995). In another study, Zhuang et al. (1995) reported that complete inactivation of *Salmonella* on tomatoes was not achieved with 320 ppm chlorine. Moreover, formation of chlorinated organic compounds, such as trihalomethanes from chlorine has raised safety concerns for their potential impact on humans and the environment, thus triggering the search for alternatives to chlorine (Parish et al., 2003).

The use of natural antimicrobial molecules for inactivating pathogenic microorganisms has received renewed attention due to concerns for toxicity of synthetic chemicals (Salamci et al., 2007). Plants have been a source of many natural molecules that contribute to human health and well-being. The antimicrobial properties of plant essential oils have been demonstrated previously (Burt, 2004), and a variety of active components in these oils has been identified. *Trans*-cinnamaldehyde (TC) is an aldehyde present as a major component of cinnamon bark extract (*Cinnamomum zeylandicum*). Carvacrol (CAR) is an antimicrobial ingredient in oregano oil obtained from *Origanum glandulosum*. Eugenol (EUG) is an active ingredient in the oil obtained from cloves (*Eugenia caryophyllis*). β -resorcylic acid (BR; 2, 4 dihydroxy benzoic acid) is widely distributed among the angiosperms, and is a secondary metabolite that plays a key role in the biochemistry and physiology of plants (Friedman et al., 2003). All these molecules are classified by the United States Food and Drug Administration as GRAS (generally recognized as safe) (Adams et al., 2004, 2005; Baskaran et al., 2010; Knowles et al., 2005).

Previous research conducted in our laboratory revealed that TC, EUG, and CAR were effective in inactivating major mastitis pathogens in milk (Ananda Baskaran et al.

2009), and TC inactivated *S. Enteritidis* and *Campylobacter jejuni* in chicken drinking water (Kollanoor Johny et al. 2008). These compounds have also been reported to possess antimicrobial activity against *S. Typhimurium* and *S. Typhimurium* DT104 (Feng et al., 2007, Si et al., 2006). Recently, we reported that TC, EUG, CAR and BR increased the sensitivity of *S. Typhimurium* DT104 to several antibiotics (Kollanoor Johny et al., 2010). The objective of this study was to determine the efficacy of TC, CAR, EUG and BR as a wash treatment for reducing *Salmonella spp.* on tomatoes.

2. Materials and Methods

2.1. Salmonella strains

The *Salmonella enterica* isolates used in this study included *S. Montevideo*, *S. Poona*, *S. Newport*, *S. Baildon*, *S. Braenderup*, and *S. Saintpaul*. These isolates from tomatoes were kindly provided by Dr. Larry B. Beuchat (Center for Food Safety, University of Georgia, Griffin, GA).

2.2. Preparation of inocula

Each *Salmonella* strain was cultured separately in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) and incubated at 37°C for 24 h with agitation (100 rpm). After three successive transfers, equal volumes of the cultures were combined and sedimented by centrifugation (3,600 x g for 15 min at 4°C). The pellet was washed two times and resuspended in phosphate buffered saline (PBS; pH 7.0) and used as the inoculum. The bacterial count in the individual cultures and the 6-strain mixture was confirmed by plating 0.1 ml portions of appropriate dilutions on tryptic soy agar (TSA, Difco) and xylose lysine desoxycholate agar (XLD; Difco) and incubating the plates at 37°C for 24 h (Kollanoor Johny et al. 2008).

2.3. Preparation and inoculation of tomatoes

Fresh plum tomatoes were purchased from a local supermarket and refrigerated for not more than 24 h. Tomatoes were equilibrated to room temperature ($23 \pm 2^{\circ}\text{C}$) before testing and washed with 70% ethanol to sterilize and remove wax residue, if there was any present. Further, the tomatoes were washed three times with sterile deionized water to remove any remaining ethanol residue and were allowed to dry in a laminar flow hood for 1 h at $23 \pm 2^{\circ}\text{C}$ under UV light before inoculation (Lang et al. 2004).

Inoculation of tomatoes was carried out according to the protocol described by Lang et al. (2004). Briefly, tomatoes were placed stem down on autoclaved aluminum foil inside a laminar flow hood. Within a 3-cm diameter circle on the surface of the blossom end of the tomato, 100 μl of the six-strain inoculum (approximately 8.0 log CFU) of *Salmonella* was applied using a micropipette. Care was taken to prevent the application of the inoculum on the blossom scar. Small and approximately equal volumes of inoculum were applied at 15 to 20 locations to help facilitate drying and prevent the inoculum from running down the side of the tomato. The inoculated tomatoes were held in a laminar flow hood to allow the inoculum to dry for 1 h at $23 \pm 2^{\circ}\text{C}$.

2.4. Treatment of tomatoes

Each tomato was placed with the blossom end down in separate 5.5" x 9" sterile sampling bags (Fisher Scientific Co LLC, Hanover Park, IL) containing 200 ml of sterile deionized water added with one of the treatments namely, 100 ppm chlorine (Burnett and Beuchat, 2002) (~ 2% NaOCl; Ricca Chemical Company, Arlington, TX), 0.5% TC (Sigma-Aldrich Corp, St. Louis, MO), 0.75% TC, 0.25% CAR (Sigma-Aldrich), 0.75% CAR, 0.25% EUG (Sigma-Aldrich), 0.75% EUG, 0.75% BR (Sigma-Aldrich) or 1.0%

BR. All plant molecules except BR were added directly to water to obtain the desired concentrations. BR powder was diluted in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) for better solubility, and added to achieve the desired concentrations. The final concentration of DMSO in dipping solutions was not more than 1%, which did not exert any antimicrobial effect (Ahameethunisa and Hopper, 2010; Nair et al. 2005; Nakamura and Hatanaka, 2002). The concentrations of CAR, TC, EUG and BR were selected based on preliminary experiments conducted in our laboratory. Treatment bags containing only sterile deionized water without any antimicrobial served as controls. In addition, inoculated, but unwashed tomatoes were also included to determine the efficiency of inoculation and obtain baseline *Salmonella* counts (baseline samples).

Each bag containing one tomato submerged in the control or treatment solution (25°C) was placed in a reciprocating water bath shaker (Model R76; New Brunswick Scientific, Edison, NJ) and shaken for 15 sec, 1 min or 3 min. At the end of each specified time, the tomato was aseptically transferred to a second bag containing 50 ml of Dey-Engley neutralizing broth (DE neutralizing broth; Difco, Becton Dickinson, Sparks, MD) (Singh et al. 2002). Each tomato was then hand-rubbed for 1 min in DE broth before determining the surviving *Salmonella* populations (Lang et al. 2004). Triplicate samples of each treatment, control and baseline were included in each experiment and the entire experiment was replicated two times.

2.5. Microbiological analyses

One ml of DE neutralizing broth from each bag was serially diluted (1:10) with 9 ml of sterile PBS, and 0.1-ml portions from appropriate dilutions were spread plated on duplicate TSA and XLD plates. A volume of 0.1-ml of wash suspension from each

sampling bag was also directly plated on duplicate TSA and XLD plates without serial dilutions. The plates were incubated at 37°C for 24 h before counting the colonies. In addition, a volume of 1 ml of wash solution from each bag was transferred to separate 250-ml Erlenmeyer flasks containing 100 ml of sterile TSB, and incubated at 37°C for 24 h. Following enrichment in TSB, the culture was streaked on XLD plates, and incubated at 37°C for 24 h. Representative colonies of bacteria from TSA and XLD plates were confirmed for *Salmonella* using the *Salmonella* rapid detection kit (Microgen Bioproducts Ltd, Camberley, UK).

2.6. Tomato color analysis

The red color of tomatoes can be measured by colorimetry and quantitatively reported as a^* values. High positive a^* values indicate more red color, while lower a^* values denote decreased red color (Batu, 2004). Four tomatoes per dipping treatment (water control, undipped control, chlorine 100 ppm, 0.25% CAR, 0.75% CAR, 0.5% TC, 0.75%TC, 0.25% EUG, 0.75% EUG, 0.75% BR and 1.0% BR; N=44) were included. Tomatoes were treated for one minute in respective treatments, and two separate a^* values were taken from each side of the tomato on day 0 and 3 of storage at 4°C using a MiniScan® EZ portable reflected-color measurement spectrophotometer (HunterLabs, Reston, VA). The values were pooled from each tomato, and averaged (n=4) to obtain the mean a^* value of tomatoes subjected to each treatment.

2.7. Statistical analyses and experimental design

Each tomato served as an experimental unit and a completely randomized 10 X 4 factorial treatment structure was followed. The factors included ten treatments (positive control, chlorine control, two concentrations each for the four plant molecules) and four

time points (0, 15 sec, 1 and 3 min) per trial. Three tomatoes were included for each treatment per time point per trial, and two independent trials were conducted. Pooled samples (n=6) were averaged and the data were analyzed using the mixed procedure of SAS (Statistical Analysis Software) ver. 9.2. Differences among the means were detected at $P < 0.05$ using the Fisher's least significance difference test with appropriate corrections for multiple comparisons. For color analysis, the data (n=4/treatment) were analyzed as mentioned above.

3. Results and Discussion

Despite considerable progress made in improving the safety of fresh fruits and vegetables, frequent outbreaks involving tomatoes continue to occur (CDC, 2008). One of the major etiological agents responsible for outbreaks in tomatoes is *Salmonella* spp. Once attached, *Salmonella* can survive on tomatoes during postharvest storage (Sapers and Jones, 2006), and is capable of growing to populations exceeding 10^7 CFU/g, provided adequate time and appropriate conditions exist (Wei et al. 1995; Zhuang et al. 1995). Thus, there is a need for developing effective ways to make tomatoes safe for human consumption. In the current study, we investigated the efficacy of four plant-derived antimicrobials namely, CAR, TC, EUG and BR added in wash solutions for reducing *Salmonella* spp. on tomatoes. Since selective culture media can potentially inhibit the recovery of antimicrobial-stressed cells of bacteria, we used the non-selective medium, TSA, and the selective agar, XLD, for enumerating *Salmonella* from treated tomatoes. However, we did not find any significant differences ($p>0.05$) between the counts of *Salmonella* recovered on the selective and non-selective agars (data not shown).

Therefore, *Salmonella* counts from the XLD plates were used for statistical analysis and discussion.

The mean population of *Salmonella* recovered from the tomatoes after inoculation (baseline) was ~ 7.0 log CFU/ml. The effect of washing tomatoes inoculated with *Salmonella spp.* in CAR and TC solutions is depicted in Fig. 1. Both concentrations of CAR decreased *Salmonella* counts on tomatoes to undetectable levels (negative by enrichment) at 1 min, and the tomatoes continued to test negative for the pathogen at 3 min. On tomatoes washed with 0.5 and 0.75% TC, *Salmonella* populations were reduced by ~ 6.0 log CFU/ml at 1 min, with complete inactivation (negative by enrichment) of the pathogen on 0.75% treated samples at 3 min of exposure. Washing of tomatoes in deionized water and chlorine for 3 min brought about ~ 2.0 and 4.0.log CFU/ml reductions in pathogen counts, respectively.

The effect of washing tomatoes in EUG and BR on *Salmonella* populations is shown in Fig. 2. All EUG and BR treatments, excluding 0.25% EUG were more effective ($p>0.05$) than water or chlorine in killing *Salmonella* on tomatoes. Washing tomatoes in water containing 0.75% EUG for 1 and 3 min reduced *Salmonella* counts by greater than 6.0 log CFU/ml (enrichment positive). Both concentrations of BR decreased the pathogen on tomatoes to undetectable levels at 3 min of exposure resulting in a 6.0 log CFU/ml reduction. Approximately 2.0 log CFU/ml and 4.0 log CFU/ml reductions in *Salmonella* counts were observed on tomatoes washed in water and chlorine for 3 min, respectively. Additional experiments were conducted to ascertain if any viable *Salmonella* were attached on the washed tomatoes, by enriching the entire fruit in 100 ml of TSB and selenite cystine broth at 37°C for 48 h. Results revealed that no *Salmonella*

were present on the tomatoes treated 0.75% CAR and 1% BR for 3 min. However, the tomatoes treated with 0.75% TC for 3 min tested positive for *Salmonella* after enrichment. With respect to surface color, there was no difference in the a^* values of tomatoes subjected to the various treatments ($P > 0.05$) (Table 1). A general trend for increasing a^* values of tomatoes was observed during storage, which could be attributed to an increase in the ripeness of the fruit (Batu, 2004).

The Food and Drug Administration proposed that antimicrobial treatments for fruits and vegetables should be capable of reducing bacterial load by a minimum of 5.0 log CFU (FDA, 2001). A variety of antimicrobial agents have been investigated for their efficacy in reducing *Salmonella* on tomatoes with varying magnitudes of bacterial reduction. These include sodium hypochlorite (Zhuang et al. 1995), ozone (Chaidez et al. 2007), and calcinated calcium (Bari et al. 2002). Among these, only calcinated calcium was effective in reducing *Salmonella* on tomatoes by greater than 5.0 log CFU (Bari et al. 2002). In addition, although plant essential oils such as sumac, oregano (Gunduz et al. 2010) and myrtle oil (Gunduz et al. 2009) were tested for killing *Salmonella* on tomatoes, these antimicrobials brought about only minimal reductions in the pathogen populations. Sumac (4%), oregano (100 ppm) and myrtle oil (1000 ppm) were reported to decrease *Salmonella* counts only by 2.38, 2.78 and 1.66 log CFU/tomato even after 20 min of washing.

Overall, the plant molecules were significantly more effective ($p < 0.05$) in reducing *Salmonella* on tomatoes compared to washing in water and chlorine. Except carvacrol, the higher concentrations of the plant molecules were generally more effective and rapid in killing *Salmonella* on tomatoes. For example, EUG at 0.75% decreased

Salmonella on tomatoes by more than 6.0 log CFU/ml after 1 min of exposure, whereas approximately 3.0 log CFU/ml of the pathogen survived on 0.25% EUG-treated samples even after 3 min. Likewise, at 1 min of exposure time, 1% BR reduced *Salmonella* to less than 1.0 log CFU/ml, whereas approximately 3.0 log CFU/ml of the pathogen was recovered from tomatoes washed with 0.75% BR. A similar concentration-dependent inactivation of *Salmonella* was observed in TC-treated tomatoes, although not as marked as with EUG and BR. Chlorine reduced the pathogen counts by ~ 4.0 log CFU/ml compared to controls, but the plant molecules brought about rapid reductions in *Salmonella*, killing large populations (greater than 6.0 log CFU/ml) even with 1 min of exposure time. After washing tomatoes, no pathogens were detected in the wash water containing the plant molecules or chlorine. However a substantial population of *Salmonella* (~ 4.0 log CFU/ml) survived in the control wash water, thus representing a potential source of cross-contamination or recontamination in case the same water is used for washing tomatoes.

Since plant derived molecules contain a number of different chemical groups in their structure, their antimicrobial activity is attributable to more than one specific mechanism (Burt, 2004). A critical property of essential oils or their components is their hydrophobicity, which helps them to target the lipid containing bacterial cell membrane (Sikkema et al. 1994). This makes these membranes more permeable leading to leakage of ions and other cell contents (Cox et al. 2006). Besides the effect on cell membranes, trans-cinnamaldehyde is also believed to kill bacteria by inhibiting energy generation and glucose uptake (Gill and Holley, 2004). Yet another mechanism by which cinnamon oil and its components kill microorganism is by their inhibitory effect on enzymes such as

amino acid decarboxylases (Wendakoon and Sakaguchi, 1995). Because of the multiple antimicrobial mechanisms, the potential for bacteria for developing resistance to plant antimicrobials is believed to be smaller (Domadia et al. 2007; Ohno et al. 2003).

In conclusion, the results of this study indicate that CAR, TC, EUG and BR were effective in rapidly reducing large populations of *Salmonella spp.* on tomatoes when used in wash water. Although CAR, TC, EUG and BR-treated tomatoes revealed no difference in their color and appearance compared to that of control samples, extensive sensory and storage studies on tomatoes treated with the plant molecules need to be completed before recommending their usage.

Acknowledgement

We thank Dr. Larry R. Beuchat at the Center for Food Safety, University of Georgia, Griffin, Georgia for providing the *Salmonella* isolates.

References

Adams, T.B., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Munro, I.C., Portoghese, P.S., Smith, R.L., Waddell W.J., Wagner, B.M., 2005. Review: The FEMA GRAS assessment of hydroxyl- and alkoxy-substituted benzyl derivatives used as flavor ingredients. *Food Chemistry and Toxicology* 43, 1241-1271.

Adams, T.B., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Munro, I.C., Portoghese, P.S., Smith, R.L., Waddell W.J., Wagner, B.M., 2004. Review: The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. *Food Chemistry and Toxicology* 42, 157-185.

Ahameethunisa, A.R., Hopper, W., 2010. Research Article: Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. *BMC Complementary and Alternative Medicine* 10, 6.

Ananda Baskaran, S., Kazmer, G.W., Hinckley, L.S., Andrew, M., Venkitanarayanan, K.S., 2009. Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens *in vitro*. *Journal of Dairy Science* 92, 1423–1429.

Bari, M.L., Inatsu, Y., Kawasaki, S., Nazuka, E., Isshiki, K., 2002. Calcinated calcium killing of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on the surface of tomatoes. *Journal of Food Protection* 65, 1706–1711.

Baskaran, Y., Periyasamy, V., Venkatraman, A.C., 2010. Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. *Toxicology* 268, 204-212.

Batu, A., 2004. Determination of acceptable firmness and color values of tomatoes. *Journal of Food Engineering* 61, 471-475.

Beuchat, L.R., 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Report WHO/FSF/FOS/98.2. World Health Organization, Geneva.

Burnett, S.L., Beuchat, L.R., 2002. Comparison of methods for fluorescent detection of viable, dead, and total *Escherichia coli* O157:H7 cells in suspensions and on apples using confocal scanning laser microscopy following treatment with sanitizers. *International Journal of Food Microbiology* 74, 37-45.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in food – a review. *International Journal of Food Microbiology* 94, 223-253.

CARD., 2004. Center for Agricultural and Rural Development. Risk free Farming? Iowa Ag Review. 10, 1-12. Accessed at: http://www.card.iastate.edu/iowaag_review/winter04/article4.aspx. Accessed on 05/28/2010.

CDC, 2008. Centers for Disease Control and Prevention. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items. United States, 2008. *Morbidity and Mortality Weekly Report* 57, 929-934.

Chaidez, C., Lopez, J., Vidales, J., Campo, N.C., 2007. Efficacy of chlorinated and ozonated water in reducing *Salmonella* typhimurium attached to tomato surfaces. *International Journal of Environmental Health Research* 17, 311–318.

Cox, N.A., Richardson, A.L.J., Buhr, A.D.R.J., Fedorka-Cray, A.P.J., Bailey, B.J.S., Wilson, B.J.L., Hiett, K.L., 2006. Natural presence of *Campylobacter* spp. in various internal organs of commercial broiler breeder hens. *Avian Diseases* 50, 450–453.

Domadia, P., Swarup, S., Bhunia, A., Sivaraman, J., Dasgupta, D., 2007. Inhibition of bacterial cell division protein FtsZ by cinnamaldehyde. *Biochemical Pharmacology* 832, 831-840.

FDA., 1998. Food and Drug Administration. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice; food labeling: warning and notice statements; labeling of juice products; proposed rules. *Federal Register* 63(79), 20450-20493.

Feng, Z., Baoping, J., Hong, Z., Hui, J., Zhiwei, Y., Jingjing, L., Jihai, L., Wenjie, Y., 2007. The antimicrobial effect of cinnamaldehyde, thymol, carvacrol and their combinations against the food borne pathogen *Salmonella* Typhimurium. *Journal of Food Safety* 27, 124-133.

Friedman, M., Henika, P.R., Mandrell, R.E., 2003. Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection* 66, 1811-1821.

Gill, A.O., Holley, R.A., 2004. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. International Journal of Food Microbiology 108, 1-9.

Gündüz, G.T., Gönül, S.A., Karapinar, M., 2009. Efficacy of myrtle oil against *Salmonella* Typhimurium on fresh produce. International Journal of Food Microbiology 130, 147-150.

Gündüz, G.T., Gönül, S.A., Karapinar, M., 2010. Efficacy of sumac and oregano in the inactivation of *Salmonella* Typhimurium on tomatoes. International Journal of Food Microbiology 141, 39-44.

Hanning, I.R., Nutt, J.D., Ricke, S.C., 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodborne Pathogens and Disease 6, 635-648.

Knowles, J.R., Roller, S., Murray, D.B., Naidu, A.S., 2005. Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureas* and *Salmonella enterica* serovar Typhimurium. Applied Environmental Microbiology 71, 797-803.

Kollanoor Johny, A., Darre, M.J., Hoagland, T.A., Schreiber, D.T., Donoghue, A.M., Donoghue, D.J., Venkitanarayanan, K.S., 2008. Antibacterial effect of *trans*-cinnamaldehyde on *Salmonella* Enteritidis and *Campylobacter jejuni* in chicken drinking water. The Journal of Applied Poultry Research 17, 490-497.

Kollanoor Johny, A., Hoagland, T.A., Venkitanarayanan, K.S., 2010. Subinhibitory concentrations of plant molecules increase the sensitivity of *Salmonella* Typhimurium DT 104 to antibiotics. *Foodborne Pathogens and Disease* 7, 1165-1170.

Lang, M.M., Harris, L.J., Beuchat, L.R., 2004. Evaluation of inoculation method and inoculum drying time for their effects on survival and efficiency of recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated on the surface of tomatoes. *Journal of Food Protection* 67, 732-741.

Nair, M.K.M., Joy, J., Vasudevan, P., Hinkley, L., Hoagland, T.A., Venkitanarayanan, K.S., 2005. Antibacterial effect of caprylic acid and monocaprylin on major bacterial mastitis pathogens. *Journal of Dairy Science* 88, 3488-3495.

Nakamura, S., Hatanaka, A., 2002. Green-Leaf-Derived C6-aroma compounds with potent antibacterial action that act on both gram-negative and gram-positive bacteria. *Journal of Agriculture and Food Chemistry* 50, 7639-7644.

Ohno, T., Kita, M., Yamaoka, Y., Imamura, S., Yamamoto, T., Mitsufuji, S., Kodama, T., Kashima, K., Imanishi, J., 2003. Antimicrobial activity of essential oils against *Helicobacter pylori*. *Helicobacter* 8, 207-215.

Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garrett, E.H., Farber, J.N., Busta, F.F., 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety* 2 (Supplement), 161-173. Accessed at: <http://onlinelibrary.wiley.com/doi/10.1111/j.1541-4337.2003.tb00033.x/pdf>. Accessed on: 10/25/2010.

Salamci, E., Kordali, S., Kotan, R., Cakir, A., Kaya, Y., 2007. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum*. *Biochemical Systematics and Ecology* 35, 569-581.

Sapers, G.M., Jones, D.M., 2006. Improved sanitizing treatments for fresh tomatoes. *Journal of food science* 71, 252-256.

Scharff, R.L., 2010. Health Related Costs from foodborne illness in the United States. Produce safety project at Georgetown University. Accessed at: <http://www.producesafetyproject.org/admin/assets/files/Health-Related-Foodborne-Illness-Costs-Report.pdf-1.pdf>. Accessed on 05/13/2010.

Si, W., Gong, J., Chanas, C., Cui, S., Yu, H., Caballero, C., Friendship, R.M., 2006. *In vitro* assessment of antimicrobial activity of carvacrol, thymol and cinnamaldehyde towards *Salmonella* serotype Typhimurium DT104: effects of pig diets and emulsification in hydrocolloids. *Journal of Applied Microbiology* 101, 1282-1291.

Sikkema, J., De Bont, J.A.M., Poolman, B., 1994. Interactions of cyclic hydrocarbons with biological membranes. *The Journal of Biological Chemistry* 269, 8022-8028.

Singh, N., Singh, R.K., Bhunia, A.K., Stroshine, R.L., 2002. Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* O157:H7 on lettuce. *Lebensmittel-Wissenschaft und-Technologie* 35, 720-729.

Sivapalasingam, S., Friedman, C.R., Cohen, L., Tauxe, R.V., 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection* 67, 2342–2353.

Wei, C.I., Huang, T.S., Kim, J.M., Lin, W.F., Tamplin, M.L., Bartz, J.A., 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. *Journal of Food Protection* 58, 829–836.

Wendakoon, C.N. Sakaguchi, M., 1995. Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. *Journal of Food Protection* 56, 410-413.

Zhuang, R.Y., Beuchat, L.R., Angulo, F.J., 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied Environmental Microbiology* 61, 2127–2131.

Figure legends

Fig. 1. Inactivation of *Salmonella spp.* on tomatoes by washing with carvacrol (CAR) and trans-cinnamaldehyde (TC) at 0, 1, 2 and 3 minutes after exposure. The treatments include Control (×), Chlorine 100 ppm (□), CAR 0.25% (◇), CAR 0.75% (◆), TC 0.5% (Δ), and TC 0.75% (▲). The detection limit is 0 CFU/ml by enrichment. Error bars represent SEM (n=6).

Fig. 2. Inactivation of *Salmonella spp.* on tomatoes by washing with eugenol (EUG) and β-resorcylic acid (βR) at 0, 1, 2 and 3 minutes after exposure. The treatments include Control (×), Chlorine 100 ppm (□), EUG 0.25% (Δ), EUG 0.75% (▲), βR 0.75% (○), and βR 1% (●). The detection limit is 0 CFU/ml by enrichment. Error bars represent SEM (n=6).

Table 1. Effect of various dipping treatments on tomato color represented as a values at 4°C (n=4/treatment) ^a

Treatments	Day 0	Day 3
Water Control	28 ± 0.6	33 ± 0.4
Chlorine	29 ± 0.5	34 ± 1.0
0.25% CAR	28 ± 0.6	28 ± 0.8
0.75% CAR	29 ± 0.6	32 ± 0.5
0.5% TC	32 ± 0.9	30 ± 0.1
0.75% TC	31 ± 1.2	32 ± 0.4
0.25% EUG	30 ± 0.8	33 ± 0.5
0.75% EUG	29 ± 0.8	32 ± 1.2
0.75% BRA	29 ± 1.0	33 ± 0.9
1.0% BRA	29 ± 1.1	31 ± 0.8
Unwashed Control	27 ± 1.2	33 ± 0.9

^a The treatments were not significantly different from each other along the columns (for day 0 or day 3) (P>0.05).

Fig. 1.

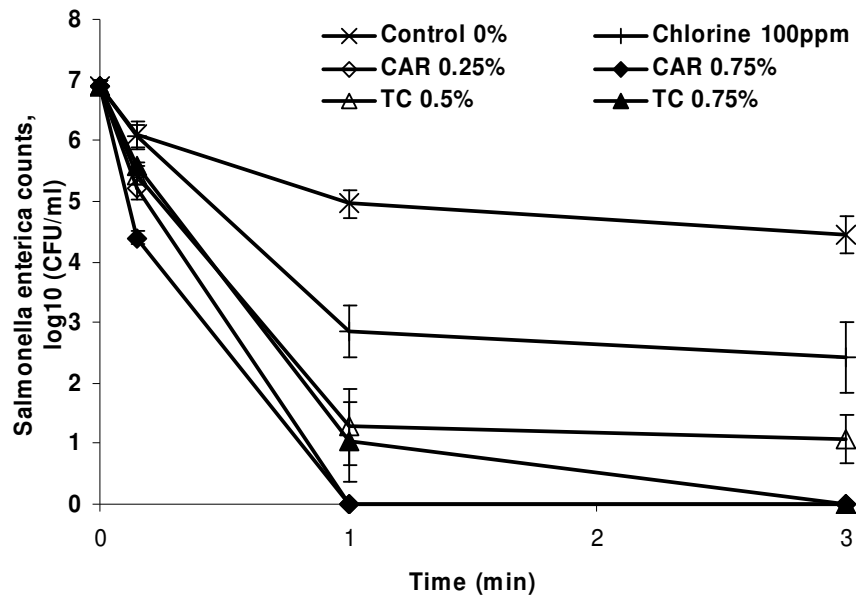
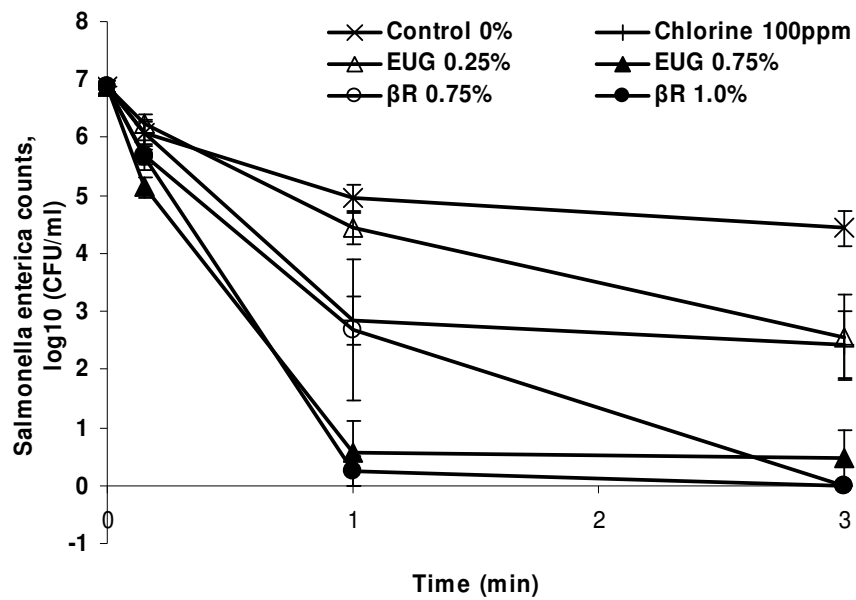


Fig. 2.



Chapter 4: Effect of trans-cinnamaldehyde and carvacrol on *Escherichia coli* O157:H7 and *Listeria monocytogenes* on iceberg lettuce

Abstract

We investigated the efficacy of two plant-derived, GRAS-status compounds, namely trans-cinnamaldehyde (TC) and carvacrol (CR) for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* on iceberg lettuce. Lettuce leaves were dip-inoculated using $\sim 10^6$ cfu/ml of a 5-strain mixture of nalidixic-acid resistant (NA^r) *E. coli* O157:H7 or NA^r *L. monocytogenes*. The lettuce leaves were air dried in a laminar airflow hood for 1 h at 23°C, and washed with sterile deionized water containing TC at 0.5% or 0.75% or CR at 0.25% or 0.75%. The leaves were transferred separately to stomacher bags containing 90 ml of neutralizing broth at 15 s, 1 min, 3 min and 5 min of washing, and stomached for 1 min. Controls included 200 ppm chlorine. Broth dilution assay were performed, and 0.1 ml of appropriate dilutions was surface-plated on TSA with NA plates, and incubated at 37°C for 24 to 48 h. Experiments were conducted with and without organic matter added to the treatment solutions. Results revealed that CR was effective in rapidly reducing both pathogens after 1 min by 3.0 to 5.0 log cfu/g in the presence and absence of organic matter. After 5 min, 0.75% CR reduced *E. coli* O157:H7 to undetectable levels. Although TC was effective in reducing *E. coli* O157:H7 by ~ 5.0 log cfu/g after 5 min in the absence of organic matter, the effect was similar to chlorine in the presence of organic matter. It is concluded that CR could potentially be added to lettuce wash solutions to reduce *E. coli* O157: H7 and *L. monocytogenes*. However, consumer acceptability studies of treated lettuce are warranted before recommending their use in wash solutions.

Keywords: plant compounds, trans-cinnamaldehyde, carvacrol, antibacterial, iceberg
lettuce

1. Introduction

Consumption of fresh produce contaminated with pathogens is a major source of foodborne outbreaks around the world (Lynch et al., 2009). In the United States, the incidence of outbreaks associated with the consumption of leafy greens has been increasing alarmingly over the last two decades. This could be attributed to the increased per capita consumption of fresh and fresh-cut fruits and vegetables in the country (Beuchat et al., 1997; Pollack, 2001; USGAO, 2002). It is estimated that the consumption of fresh market romaine and leaf lettuce increased from 10.6 lbs in 2005 to 15.1 lbs in 2008 (Tables for Fresh Vegetables, 2009). Of the fresh produce consumed in the U.S., lettuce is perceived by consumers as healthful and nutritious, and is one of the most commonly consumed leafy greens (USDA ERS, 2013). The United States remains a leading lettuce exporter, second behind Spain. U.S. exports of all types of lettuce was 327,268 metric tons (MT) in 2010 valued at \$439.3 million (FAS, 2011).

A major concern associated with the increase in the consumption of fresh produce is the potential risk of foodborne diseases. In the United States alone, the proportion of outbreaks linked to fresh produce increased from less than 1% of all reported outbreaks with a known food vehicle in the 1970s to 6% in the 1990s (Sivapalasingam et al., 2004). Leafy greens such as lettuce (all varieties) were identified as the commodity group of highest concern from a microbiological safety perspective, and regarded as level 1 priorities (FAO/WHO, 2008). Foodborne pathogens, *E. coli* O157: H7 and *Listeria monocytogenes* were implicated in several outbreaks involving leafy green vegetables, including lettuce (Hilborn, 1999; CDC, 2006; FDA, 2006; Falkenstein, 2010; FDA, 2010). Among the various sources, contaminated soil, water used for irrigation, ice, fresh

produce slicers, shredders, and food handlers play a role in the pre- and post-harvest contamination of lettuce (reviewed by Obaidat et al., 2009).

Chlorine is commonly used for the decontamination of fresh produce (Gunduz et al., 2009; Zhang et al., 2009). Washing produce with sodium-chlorinated water (NaClO) is the most commonly used method to remove pathogens from fruit and vegetable surfaces. Wash water containing 50–200 ppm of chlorine is widely used in processing plants to decontaminate fresh produce received from the farm (Gunduz et al., 2009). Studies have shown that treatment with chlorine generally resulted in a reduction of bacterial populations of 1 to 2 log cfu/g on fruits and vegetables (Weissinger et al., 2000; Beuchat et al., 2001; Beuchat et al., 2004; Burnett et al., 2004). Chlorine has a limited effect on fresh produce due to its reduced bactericidal effect in the presence of organic matter (Obaidat et al., 2009). Moreover, active hypochlorite could react with nitrogen-containing compounds in foods, resulting in potentially toxic halogenated organic compounds (Wei et al., 1985).

The use of natural antimicrobial molecules for inactivating pathogenic microorganisms has received renewed attention due to concerns for toxicity of synthetic chemicals (Salamci et al., 2007). Plants have been the source of many molecules that contribute to human health and well-being. The active components in several herbal and traditional plant-derived medicines are believed to play a role in improving health (Wollenweber, 1988). The antimicrobial properties of plant essential oils have been demonstrated previously (Burt, 2004; Holley et al., 2005), and a variety of active components in these oils has been identified. *Trans*-cinnamaldehyde (TC) is an aldehyde present as a major component of cinnamon bark extract (*Cinnamomum zeylandicum*)

(reviewed by Burt, 2004). Carvacrol (CR) is an antimicrobial ingredient in oregano oil obtained from *Origanum glandulosum* (Bendahou et al., 2008). Both of these molecules are considered to be GRAS chemicals by the FDA (TC – 21 CFR 182.60; CR – 21 CFR 172.515).

The objective of the current study was to determine the efficacy of TC and CR in inactivating *E. coli* O157:H7 and *L. monocytogenes* on iceberg lettuce in the presence and absence of organic matter.

2. Material and methods

2.1. Bacterial strains and preparation of inoculum

Five strains of *E. coli* O157:H7 or *L. monocytogenes* were used. The strains were preinduced for resistance to nalidixic acid (NA; Sigma-Aldrich, St. Louis, MO) at 50 µg/ml for selective enumeration. Strains were cultured separately in 10 ml of tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) supplemented with 50 µg/ml NA and incubated at 37°C for 24 h with agitation (100 rpm). After three successive transfers, equal volumes of the cultures were combined and sedimented by centrifugation (3,600 g for 15 min at 4°C). The pellet was resuspended in autoclaved deionized water and used as the inoculum. The bacterial count of the individual cultures and the five-strain mixture were confirmed by plating 0.1-ml portions of appropriate dilutions on tryptic soy agar (TSA; Difco, Becton Dickinson) plates containing NA (TSA-NA) and incubating the plates at 37°C for 24 h.

2.2. Preparation and inoculation of lettuce

Lettuce leaves were prepared and inoculated according to previously published protocols (Beuchat et al., 1995; Li et al., 2002; Singh et al., 2002). Iceberg lettuce

(*Lactuca sativa* L.) was purchased from a local supermarket and stored at 4°C and used within 24 h of purchase. The outer three or four leaves and core were removed from the head of the lettuce and discarded. Lettuce leaves were then cut into 10 g portions and were equilibrated to room temperature (23 ± 2 °C) before use. A mixture containing equal populations of *L. monocytogenes* or *E. coli* O157:H7 was added to 1 liter of autoclaved deionized water so that the inoculum level on each lettuce leaf was ~ 6.0 log cfu/g. To obtain the required inoculum the lettuce leaves were immersed in the solution for approximately 1 min with agitation in a reciprocal water bath shaker (model R76; New Brunswick Scientific, Edison, NJ). They were drained and held in a laminar flow hood to dry for 1 h at 23 ± 2 °C.

2.3. Preparation of organic load

The method for preparation of the organic matter was similar to that used by Zhang et al (2009). Two to three outer layers of the lettuce leaves were removed and discarded. Two hundred g of leaves were placed in a sterile blender jar with 200 ml of sterile water tempered to 23 ± 2 °C and were blended evenly on high speed. The organic load was prepared immediately before use.

2.4. Treatment solutions

Lettuce leaves were immersed in sterile stainless steel bowls containing 500 ml of sterile deionized water or 450 ml sterile deionized water plus 50 ml of organic load (prepared as mentioned earlier) containing one of the treatments namely, chlorine (200 µg/ml) (2% NaOCl; Ricca Chemical Company, Arlington, TX), 0.5% TC (Sigma-Aldrich Corp, St. Louis, MO), 0.75% TC, 0.25% CR (Sigma-Aldrich Corp, St. Louis, MO), and 0.75% CR. Treatments containing added organic contaminant were also included for each

trial. The bowls were placed in a reciprocating water bath shaker and agitated for 15 sec, 1 min, 3 min or 5 min. Each leaf was aseptically transferred to a bag containing 40 ml of Dey-Engley neutralizing broth (DE neutralizing broth; Difco, Becton Dickinson, Sparks, MD) (Beuchat et al., 2004) and was stomached (Stomacher 400 Lab Blender; Seward Medical Ltd, London, UK) for 1 min.

2.5. Microbiological analyses

Populations (cfu/g) of *E. coli* O157: H7 and *L. monocytogenes* were determined using broth dilution assay. The samples in the neutralizing broth were mixed vigorously and serially diluted 10-fold in sterile PBS. A 0.1 ml portion of appropriate dilutions were surface plated on triplicate TSA-NA agar plates (TSA; Difco, Becton Dickinson, Sparks, MD). Colonies were enumerated after incubation at 37°C for 24 to 48 h. When colonies were not detected by direct plating, samples were tested for surviving *L. monocytogenes* and *E. coli* O157: H7 by enrichment for 48 h at 37°C in 100 ml TSB followed by streaking on TSA-NA plates. Representative colonies from TSA-NA plates were confirmed as *L. monocytogenes* and *E. coli* O157 by plating on Oxford agar with supplement (OX; Difco, Becton Dickinson, Sparks, MD), or Sorbitol McConkey agar (SMA; Difco, Becton Dickinson, Sparks, MD), respectively.

2.6. Un-inoculated lettuce leaves (negative control)

Un-inoculated lettuce leaves (three samples per experiment) were analyzed for the presence of any inherent *L. monocytogenes* or *E. coli* O157: H7. Each lettuce leaf was placed in 40 ml of DE neutralizing broth and stomached for 1 min, diluted and plated on Oxford and SMA agar plates. The plates were incubated at 37°C for 24 to 48 h. One ml of neutralizing broth was transferred aseptically to 100 ml of TSB for enrichment by

incubating at 37°C for 48 h before streaking on Oxford and SMA agar plates for selective identification.

2.7. Determination of baseline bacteria

Inoculated but untreated (control) lettuce leaves (three samples per experiment) were used to determine the *L. monocytogenes* and *E. coli* O157: H7 populations (cfu/g) attached to the lettuce leaves after inoculation and drying, in order to obtain a base-line.

2.8. Statistical analysis

Each lettuce leaf served as an experimental unit, and a completely randomized design with 12 x 4 treatment structure was followed for each bacteria. The factors included 12 treatments (0%, 200 ppm chlorine, 0.5% TC, 0.75% TC, 0.25% CR, and 0.75% CR, with and without organic matter), and 4 time points (15s, 1min, 3min and 5min). Each treatment was duplicated and the experiment was repeated three times. The data were pooled and analyzed using the PROC-MIXED procedure of SAS (Ver. 9.2, Statistical Analysis Software, Cary, NC). The differences between the means were considered significant at $P < 0.05$ and were detected using Fisher's least significance difference test with appropriate corrections for multiple comparisons. Each experiment was replicated three times.

3. Results

3.1. The effect of plant-molecules on E. coli O157:H7

3.1.1. Effect of CR in sterile deionized water

The effect of CR on *E. coli* O157:H7 on lettuce in the absence of organic matter is shown in figure 1. After 1 min, 0.75% CR rapidly reduced pathogen populations resulting in approximately a 2.5 log cfu/g reduction, whereas 0.25% CR reduced the pathogen by

approximately 1.0 log cfu/g of lettuce leaf, compared to control ($P < 0.05$). However, chlorine at 200 ppm did not reduce the pathogen, compared to the controls. After 3 and 5 min, 0.75% CR was able to reduce pathogen populations to undetectable levels resulting in a reduction of 6.0 log cfu/g. The lower concentration of CR, 0.25% resulted in 2.0 log cfu/g reduction of *E. coli* O157: H7. On the other hand, chlorine reduced the pathogen populations only minimally after 3 and 5 min (Fig 1).

3.1.2. Effect of TC in sterile deionized water

The effect of TC on *E. coli* O157:H7 on lettuce in the absence of organic matter is shown in figure 2. After 15 sec, 0.75% TC had a 1.0 to 1.5 log cfu/g reduction, compared to the control ($P < 0.05$). However at 1, 3 and 5 min 0.75% TC reduced *E. coli* O157: H7 populations significantly by 2.5, 3.5 and 5.0 log cfu/g whereas, 0.5% TC reduced the pathogen population by approximately 1.5, 2.0 and 2.5 log cfu/g during those time periods, respectively. At 1, 3 and 5 min 200 ppm chlorine was only able to reduce the pathogen population by ~1.0 log cfu/g, compared to the control (Fig 2).

3.1.3. Effect of CR in the presence of organic matter

The effect of CR on *E. coli* O157:H7 on lettuce in the presence of organic matter is shown in figure 3. After 15 sec, 0.75% CR was able to rapidly reduce pathogen populations by approximately a log cfu/g ($P < 0.05$), compared to the control. The magnitude of reduction was larger after 1 min since 0.75% CR could rapidly reduce the pathogen to > 5.0 log cfu/g, and later after 5 min to ~6 log cfu/g ($P < 0.05$). The lower concentration of CR, 0.25% was able to reduce the pathogen significantly by ~2.0 to 2.5 log cfu/g after 3 and 5 min, compared to the control.

3.1.4. Effect of TC in the presence of organic matter

The effect of TC on *E. coli* O157:H7 on lettuce in the presence of organic matter is depicted in Figure 4. None of the treatments differed from each other ($P > 0.05$). The results showed that TC was not effective in reducing *E. coli* O157:H7 in the presence of organic matter.

3.2. The effect of plant molecules on L. monocytogenes

3.2.1. Effect of CR in sterile deionized water

The effect of CR on *L. monocytogenes* on lettuce in the absence of organic matter is shown in figure 5. At 15 sec, 0.75% CR was able to rapidly reduce the pathogen load by approximately 2.0 log cfu/g ($P < 0.05$), while 0.25% CR was similar to that of chlorine in effect. However, after 1, 3 and 5 min, the magnitude of reduction brought about by 0.75% CR was increased by 4.5, 5.5 and 5.5 log cfu/g respectively, compared to the control ($P < 0.05$). After 3 and 5 min, 0.25% CR reduced the pathogen load by one log as observed in chlorine treatment (Fig 5).

3.2.2. Effect of TC in sterile deionized water

The efficacy of TC in decreasing *L. monocytogenes* on lettuce in the absence of organic matter can be observed in Figure 6. The inhibitory effect of TC was very minimal on *L. monocytogenes* on lettuce. No significant differences were observed among the treatments at any of the sampling time point, compared to control ($P > 0.05$).

3.2.3. Effect of CR in the presence of organic matter

The effect of CR on *L. monocytogenes* on lettuce in the presence of organic matter is shown in figure 7. After 15 sec, 1, 3 and 5 min, 0.75% CR rapidly reduced the pathogen population by 1.0, 3.0, 4.0 and 4.0 log cfu/g, respectively compared to control

($P < 0.05$). After 5 min, 0.25% CR reduced the pathogen by approximately 2.0 log cfu/g, compared to chlorine ($P < 0.05$).

3.2.4. Effect of TC in the presence of organic matter

The effect of TC on *L. monocytogenes* on lettuce in the presence of organic matter is provided in Figure 8. No significant differences were observed among the treatments at any of the time points ($P > 0.05$). The results show that the concentrations of TC used in this study are not effective in reducing *L. monocytogenes* in the presence of organic matter.

4. Discussion

This study determined the efficacy of two GRAS-status, plant-derived antimicrobial compounds for reducing *E. coli* O157: H7 and *Listeria monocytogenes* on iceberg lettuce. Given the elevated numbers of foodborne outbreaks associated with consumption of contaminated leafy greens, and the reduced efficacy of chlorine compounds in the presence of organic matter, there is a critical need for alternate decontaminating methods to ensure produce safety for consumers.

Spraying, washing or dipping of fresh produce with several combinations of acids and sanitizers is the common disinfection practice followed in the industry (Beuchat, 1998; Zhang et al., 2009). The major treatments recommended within industry consist of the use of chlorine (chlorine gas, sodium hypochlorite, calcium hypochlorite and chlorine dioxide), hydrogen peroxide, tri-sodium phosphate, quaternary ammonium compounds, ozone, ionizing irradiation, acids, and organic acids (lactic acid and citric acid) (Beuchat, 1998). The efficacy of chlorine compounds in disinfecting fresh produce, including leafy greens, has been previously investigated by several researchers. For example, Delaquis et

al. (2002) inoculated cut iceberg lettuce with *E. coli* O157:H7 and *L. monocytogenes* before washing for 3 min in cold (4°C) (current method used in industry) and warm (47°C) water containing 100 ppm total chlorine, and enumerated surviving populations of the bacteria. It was observed that *E. coli* O157:H7 and *L. monocytogenes* populations declined over time when washed with cold water containing chlorine and during subsequent storage for up to 14 days. However, the pathogen population increased over time when washed with warm water and chlorine. In yet another study, Li et al. (2001) studied the survival and growth of *E. coli* O157:H7 on lettuce treated with 20 ppm chlorine at either 20 or 50°C, followed by subsequent storage at 5°C for 18 days or at 15°C for 7 days. They reported that the pathogen population declined throughout storage at 5°C, but increased by 2.3 to 3.2 log cfu/g within 2 days at 15°C. In addition to the moderate efficacy of chlorine on inactivation of the aforementioned pathogens, the active hypochlorite could lose its efficacy by reacting with nitrogen containing compounds in foods, resulting in the formation of halogenated organic compounds (Wei et al., 1985). Fawell (2000) reported that chlorine can react with naturally occurring organic matter to form chloroform and other trihalomethanes such as bromodichloromethane, chlorodibromomethane and bromoform. These compounds, especially trihalomethanes, have been shown to increase tumors of the liver, kidney or large intestine in rats and mice (Fawell, 2000) and are toxic to humans (Chen et al., 2003).

Results from the current study demonstrated that regardless of the presence or absence of organic matter, carvacrol is effective in rapidly inactivating *E. coli* O157: H7 and *L. monocytogenes* on lettuce. For example, after one minute of treatment in the presence and absence of organic matter, 0.75% CR was able to reduce *E. coli* O157:H7

populations by approximately 5.0 log cfu/g and to undetectable levels after 5 min (Figs 1, 3). In addition, 0.75% CR reduced *L. monocytogenes* counts by 3.0 – 4.5 log cfu/g after 1 min and by more than 5.0 log cfu/g after 5 minutes in the presence and absence of organic matter (Figs. 5, 7). Carvacrol was effective against both pathogens, compared to chlorine. The higher concentration of TC was able to reduce *E. coli* O157:H7 by approximately 5.0 log cfu/g after 5 min in sterile deionized water (Fig. 2). However, TC had no significant antimicrobial effect on either *L. monocytogenes* or *E. coli* O157:H7 on lettuce, compared to the control or chlorine in the presence of organic matter (Figs. 4, 8). Moreover, TC was not effective in significantly reducing *L. monocytogenes* even in the absence of organic matter (Fig. 6). Viazis et al. (2011) observed a similar reduced antimicrobial effect of TC on *E. coli* O157: H7 in tryptic soy broth (without organic contamination) placed on baby romaine lettuce leaves at 23°C. Treatment with TC brought about ~2 log reduction of the pathogen on romaine lettuce.

To conclude, the results indicate that CR was effective in rapidly reducing large populations of *L. monocytogenes* and *E. coli* O157:H7 on lettuce when used in wash water even in the presence of organic matter. However, extensive sensory and storage studies on lettuce treated with CR need to be completed before recommending its usage.

References

- Abe, T., Crooked, L., Hill, C., 1995. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology*, 28, 169–185.
- Bendahou, M., Muselli, A., Grignon-Dubois, M., Benyoucef, M., Desjobert, J., Bernardini, A., Costa, J., 2008. Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. essential oil and extract obtained by microwave extraction: Comparison with hydrodistillation. *Food Chemistry*, 106, 132–139.
- Beuchat, L. R., 1998. Surface decontamination of fresh fruits and vegetables eaten raw: A Review. Accessed at:
http://www.who.int.ezproxy.lib.uconn.edu/foodsafety/publications/fs_management/en/surface_decon.pdf Accessed on: 4/19/2009
- Beuchat, L. R., Doyle, M. P., 1995. Survival and growth of *Listeria monocytogenes* in foods treated or supplemented with carrot juice. *Food Microbiology*, 12, 73-80.
- Beuchat, L. R., Ryu, J. H., 1997. Produce handling and processing practices. *Emerging Infectious Diseases*, 3, 459–465.
- Beuchat, L. R., Adler, B. B., Lang, M. M., 2004. Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. *Journal of Food Protection*, 67, 1238–1242.
- Beuchat, L. R., Farber, J. M., Garrett, E. H., Harris, L. J., Parish, M. E., Suslow, T. V., Busta, F. F., 2001. Standardization of a method to determine the efficacy of

- sanitizers in inactivating human pathogenic microorganisms on raw fruits and vegetables. *Journal of Food Protection*, 64, 1079–1084.
- Beuchat, L. R., Lang, M. M., & Harris, L. J., 2004. Evaluation of inoculation method and inoculum drying time for their effects on survival and efficiency of recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated on the surface of tomatoes. *Journal of Food Protection*, 67, 732-741.
- Burnett, A. B., Iturriaga, M. H., Escartin, E. F., Pettigrew, C. A., Beuchat, L. R., 2004. Influence of variations in methodology on populations of *Listeria monocytogenes* recovered from lettuce treated with sanitizers. *Journal of Food Protection*, 67, 742–750.
- Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in food – a review. *International Journal of Food Microbiology*, 94, 223–253.
- Carson, C. F., Mee, B. J., Riley, T. V., 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrobial Agents and Chemotherapy*, 46, 1914-1920.
- Chen, J., Douglas, G. C., Thirkill, T. L., Lohstroh, P. N., Bielmeier, N. R., Narotsky, M. G., Best, D. S., Harrison, R. A., Natarajan, K., Pegram, R. A., Overstreet, J. W., Lasley, B. L., 2003. Effect of Bromodichloromethane on Chorionic Gonadotrophin Secretion by Human Placental Trophoblast Cultures. *Toxicological Sciences*, 76, 75– 82.
- Cox, N. A., Richardson, A. L. J., Buhr, A. D. R. J., Fedorka-Cray, A. P. J., Bailey, B. J. S., Wilson, B. J. L., Hiatt, K. L., 2006. Natural presence of *Campylobacter* spp. in

- various internal organs of commercial broiler breeder hens. *Avian Diseases*, 50, 450–453.
- Delaquis, P., Stewart, S., Cazaux, S., Toivonen, P., 2002. Survival and growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in ready-to-eat iceberg lettuce washed in warm chlorinated water. *Journal of Food Protection*, 65, 459–464.
- Domadia, P., Swarup, S., Bhunia, A., Sivaraman, J., Dasgupta, D., 2007. Inhibition of bacterial cell division protein FtsZ by cinnamaldehyde. *Biochemical Pharmacology*, 832, 831-840.
- FAO/WHO. (2008). Microbiological hazards in fresh fruits and vegetables. Microbiological risk assessment series – Meeting Report, 19–21 October 2007. Agriculture Organization of the United Nations/World Health Organization Accessed at: http://www.who.int/foodsafety/publications/micro/MRA_FruitVeges.pdf Accessed on 2/14/2011.
- Fawell, J., 2000. Risk assessment case study – chloroform and related substances. *Food and Chemical Toxicology*, 38, 91-95.
- Foreign Ag Service (FAS), Global Agricultural Trade System (GATS), United States Department of Agriculture (USDA), 2011.
- Gill, A. O., Holley, R. A., 2004. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *International Journal of Food Microbiology*, 108, 1-9.
- Gunduz, G. T., Aktug, S. A., Karapinar, G. M., 2009. Efficacy of oregano oil in the inactivation of *Salmonella* Typhimurium on lettuce. *Food Control*, 21, 513–517.

- Holley, R. A., Patel, D., 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *International Journal of Food Microbiology*, 22, 273–292.
- Knobloch, K., Weigand, H., Weis, N., Schwarm, H. M., Vigneschow, H., 1986. Action of terpenoids on energy metabolism. In Brunke, E. J. (Ed.), *Progress in essential oil research*, 16th International symposium on essential oils (pp. 429– 445). Berlin: De Gruyter.
- Li, Y., Brackett, R. E., Chen, J., Beuchat, L. R., 2001. Survival and growth of *Escherichia coli* O157:H7 inoculated onto cut lettuce before or after heating in chlorinated water, followed by storage at 5 or 15°C. *Journal of Food Protection*, 64, 305–309.
- Li, Y., Brackett, R. E., Chen, J., Beuchat, L. R., 2002. Mild heat treatment of lettuce enhances growth of *Listeria monocytogenes* during subsequent storage at 5°C or 15°C. *Journal of Applied Microbiology*, 92, 269-274.
- Lynch, M., Tauxe, R., Hedberg, C., 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection*, 137, 307-315.
- Obaidat, M. M., Frank, J. F., 2009. Inactivation of *Escherichia coli* O157: H7 on the intact and damaged portions of lettuce and spinach leaves by using allyl isothiocyanate, carvacrol, and cinnamaldehyde in vapour phase. *Journal of Food Protection*, 72, 2046-2055.

- Ohno, T., Kita, M., Yamaoka, Y., Imamura, S., Yamamoto, T., Mitsufuji, S., Kodama, T., Kashima, K., Imanishi, J. (2003). Antimicrobial activity of essential oils against *Helicobacter pylori*. *Helicobacter*, 8, 207-215.
- Pollack, S., 2001. Consumer demand for fruit and vegetables: the U.S. example Accessed at: <http://www.ers.usda.gov/publications/wrs011/wrs011h.pdf> Accessed on: 5/10/2010
- Salamci, E., Kordali, S., Kotan, R., Cakir, A., Kaya, Y., 2007. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum*. *Biochemical Systematics and Ecology*, 35, 569-581.
- Sikkema, J., De Bont, J. A. M., & Poolman, B., 1994. Interactions of cyclic hydrocarbons with biological membranes. *The Journal of Biological Chemistry*, 269, 8022-8028.
- Singh, S., Singh, R. K., Bhunia, A. K., Stroshine, R. L., 2002. Efficacy of chlorine dioxide, ozone and thyme essential oil of a sequential washing in killing *E. coli* O157:H7 on lettuce and baby carrots. *Lebensmittel-Wissenschaft und Technologie*, 35, 720-729.
- Sivapalasingam, S., Friedman, C. R., Cohen, L., Tauxe, R. V., 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection*, 67, 2342-2353.
- Skandamis, P. N., Koutsoumanis, K., Fasseas, K., Nychas, G. J. E., 2001. Inhibition of oregano oil and EDTA on *Escherichia coli* O157: H7. *Italian Journal of Food Science*, 13, 65-75.

- USDA ERS, 2013. Ag and Food statistics: Charting the essentials. Available at <http://www.ers.usda.gov/data-products/ag-and-food-statistics-charting-the-essentials.aspx#.U0gF1F4t1LY>.
- USGAO, 2002. Fruits and vegetables: enhanced federal efforts to increase consumption could yield health benefits for Americans. Accessed at: <http://www.gao.gov/new.items/d02657.pdf> Accessed on: 5/10/2010.
- Ultee, A., Kets, E. P. W., Smid, E. J., 1999. Mechanisms of action of carvacrol on the food-borne-pathogen *Bacillus cereus*. Applied Environmental Microbiology, 65, 4606-4610.
- Viazis, S., Akhtar, M., Feirtag, J., Deiz-Gonzalez, F., 2011. Reduction of *Escherichia coli* O157: H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. Food Microbiology, 28, 149-157.
- Wei, C. I., Cook, D. L., Krik, J. R., 1985. Use of chlorine compounds in the food industry. Food Technology 39,107–115.
- Weissinger, W. R., Chantarapanont, W., Beuchat, L. R., 2000. Survival and growth of *Salmonella bairdii* in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. International Journal of Food Microbiology, 62, 123–131.
- Wendakoon, C. N., Sakaguchi, M., 1995. Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. Journal of Food Protection, 56, 410-413.

Wollenweber, E., 1988. Occurrence of flavonoid aglycones in medicinal plants. Progress in Clinical and Biological Research, 280, 45-55.

Zhang, G., Ma, L., Phelan, V. H., Doyle, M. P., 2009. Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157:H7. Journal of Food Protection 72, 1392-1397.

Figure Captions

Fig 1. Effect of carvacrol on *E. coli* O157: H7 on iceberg lettuce in sterile deionized water at 23°C

Fig 2. Effect of trans-cinnamaldehyde on *E. coli* O157: H7 on iceberg lettuce in sterile deionized water at 23°C

Fig 3. Effect of carvacrol on *E. coli* O157: H7 on iceberg lettuce in the presence of organic matter at 23°C

Fig 4. Effect of trans-cinnamaldehyde on *E. coli* O157: H7 on iceberg lettuce in the presence of organic matter at 23°C

Fig 5. Effect of carvacrol on *L. monocytogenes* on iceberg lettuce in sterile deionized water at 23°C

Fig 6. Effect of trans-cinnamaldehyde on *L. monocytogenes* on iceberg lettuce in sterile deionized water at 23°C

Fig 7. Effect of carvacrol on *L. monocytogenes* on iceberg lettuce in the presence of organic matter at 23°C

Fig 8. Effect of trans-cinnamaldehyde on *L. monocytogenes* on iceberg lettuce in the presence of organic matter at 23°C

Fig. 1

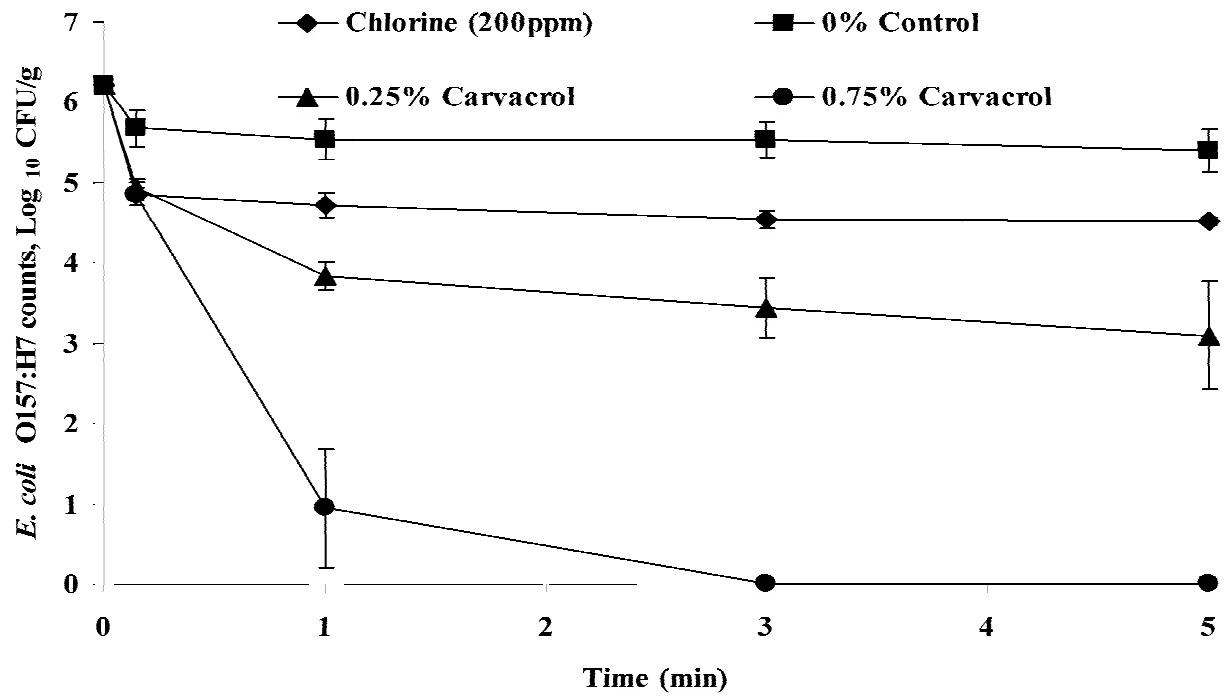


Fig. 2

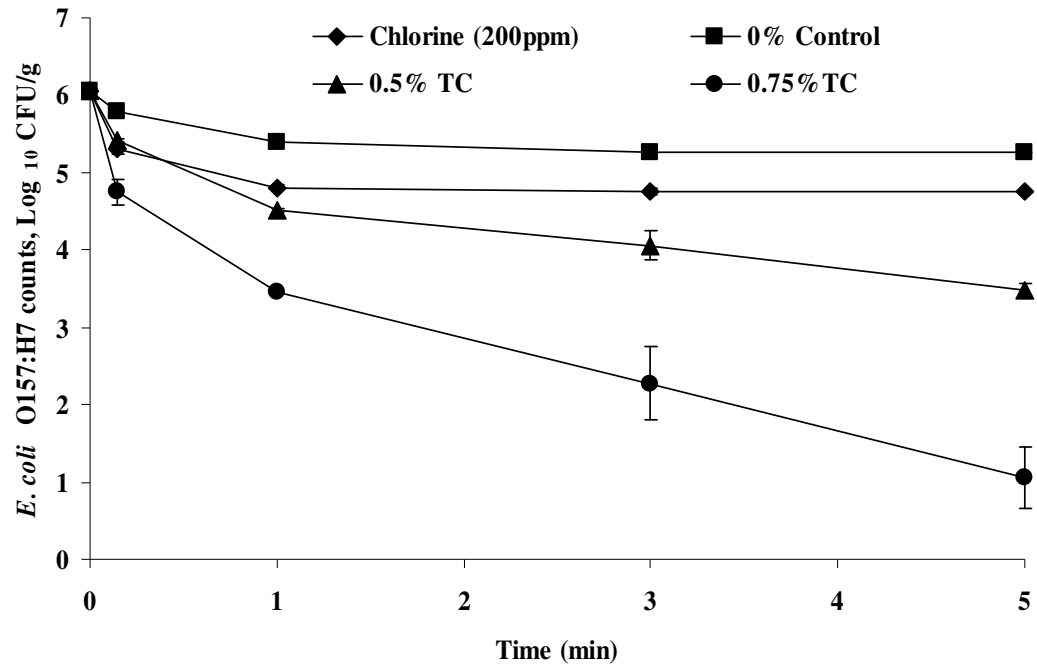


Fig. 3

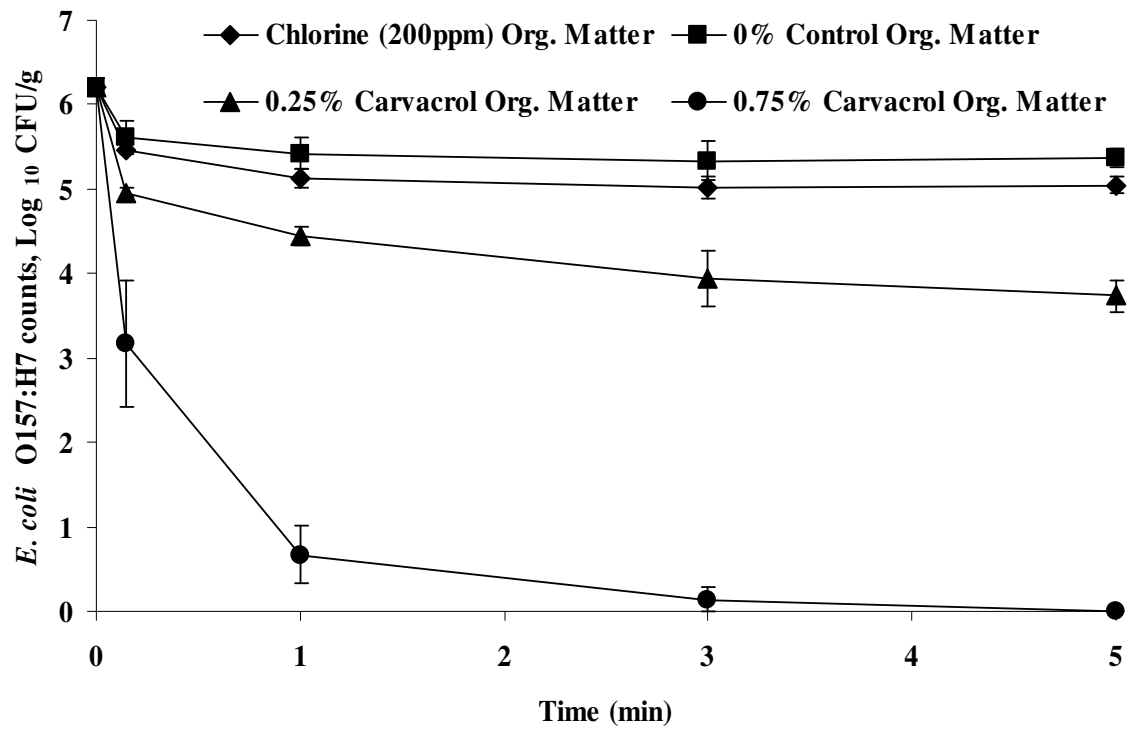


Fig. 4

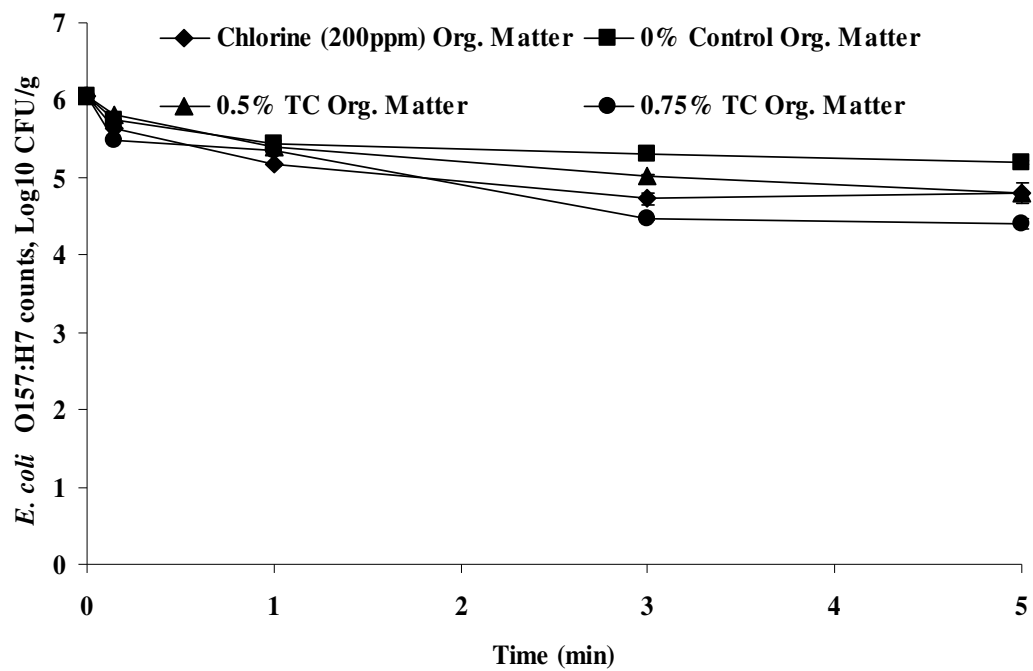


Fig. 5

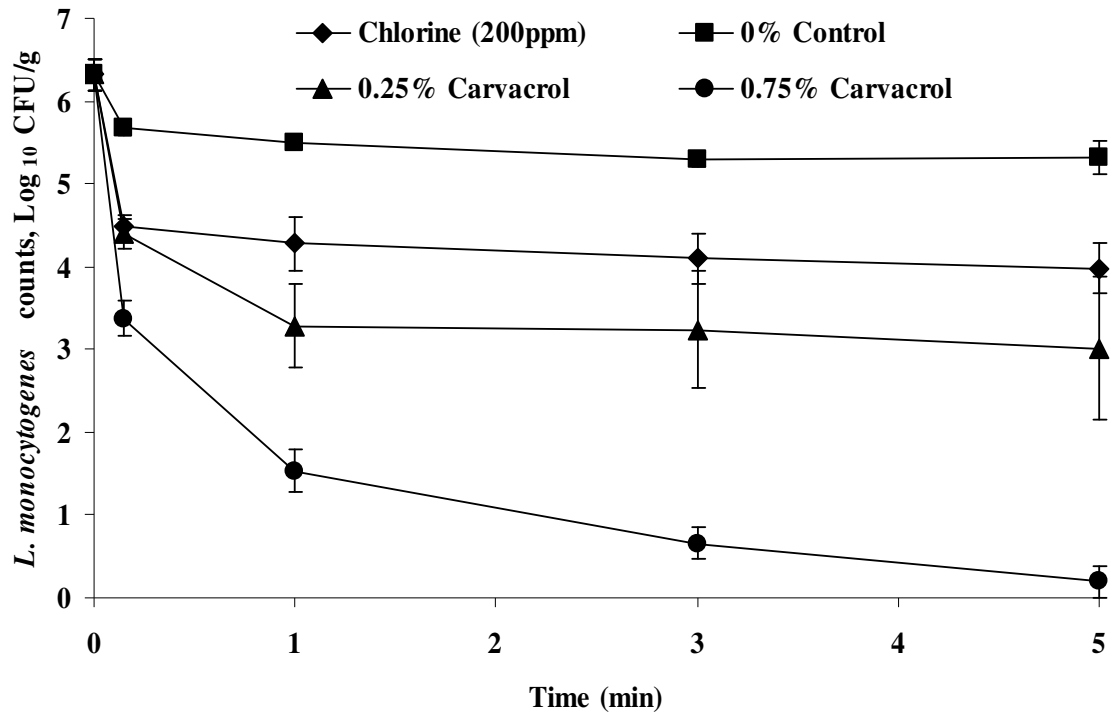


Fig. 6

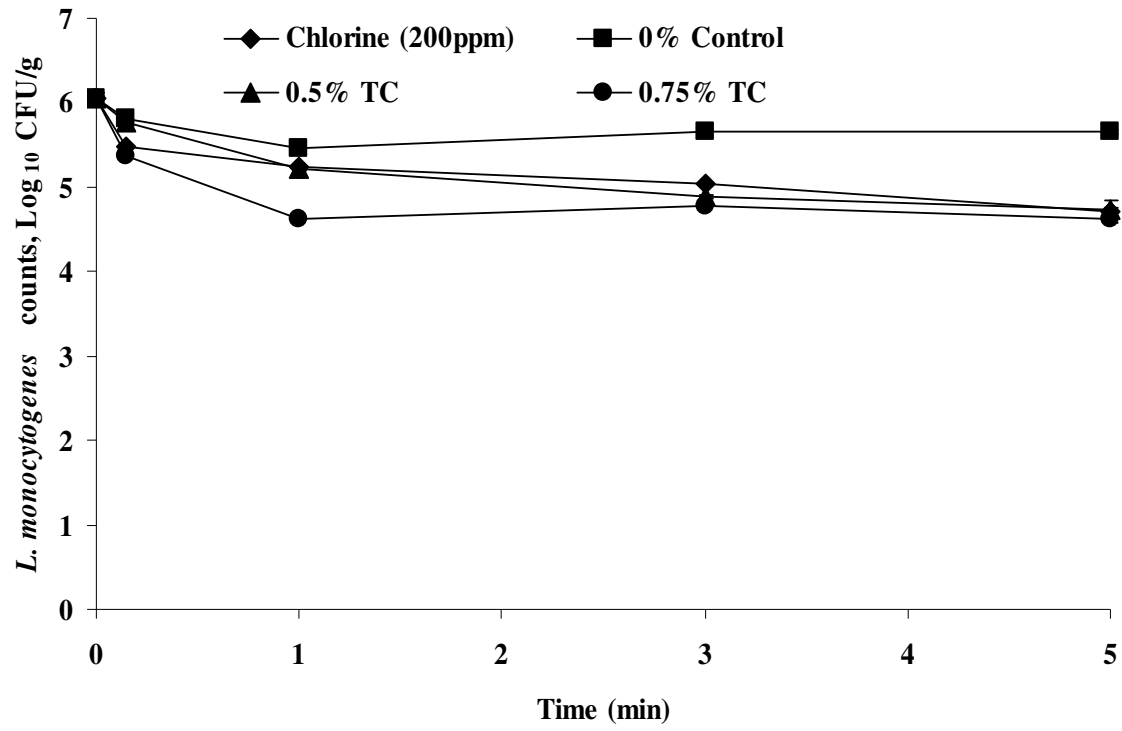


Fig. 7

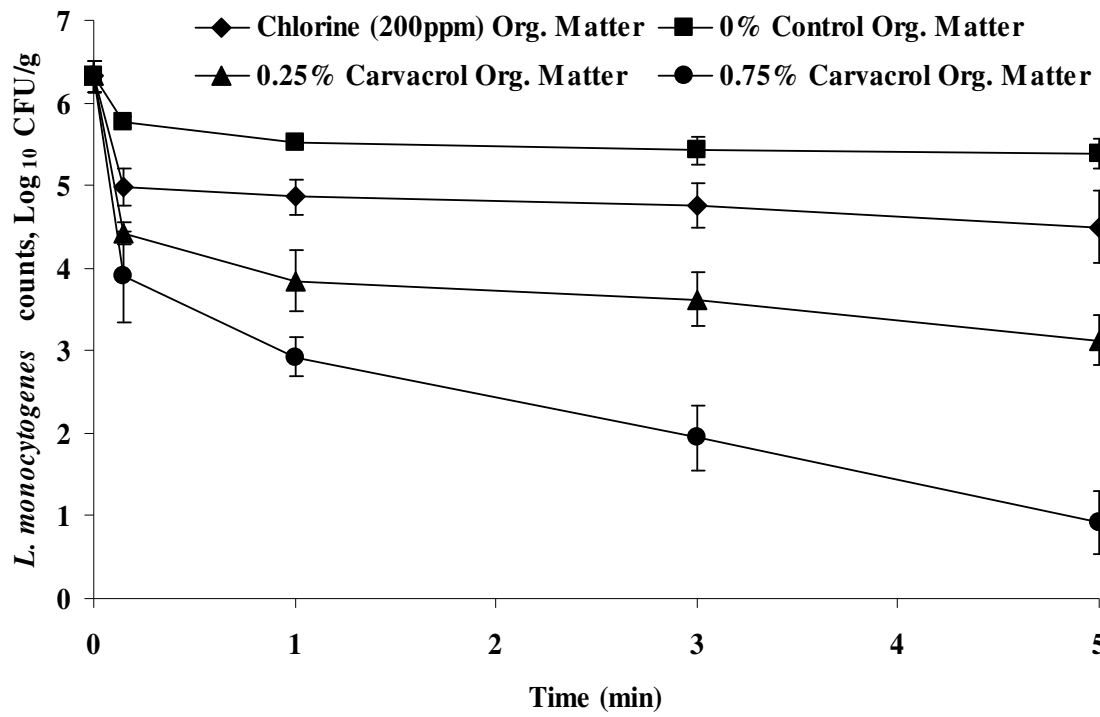
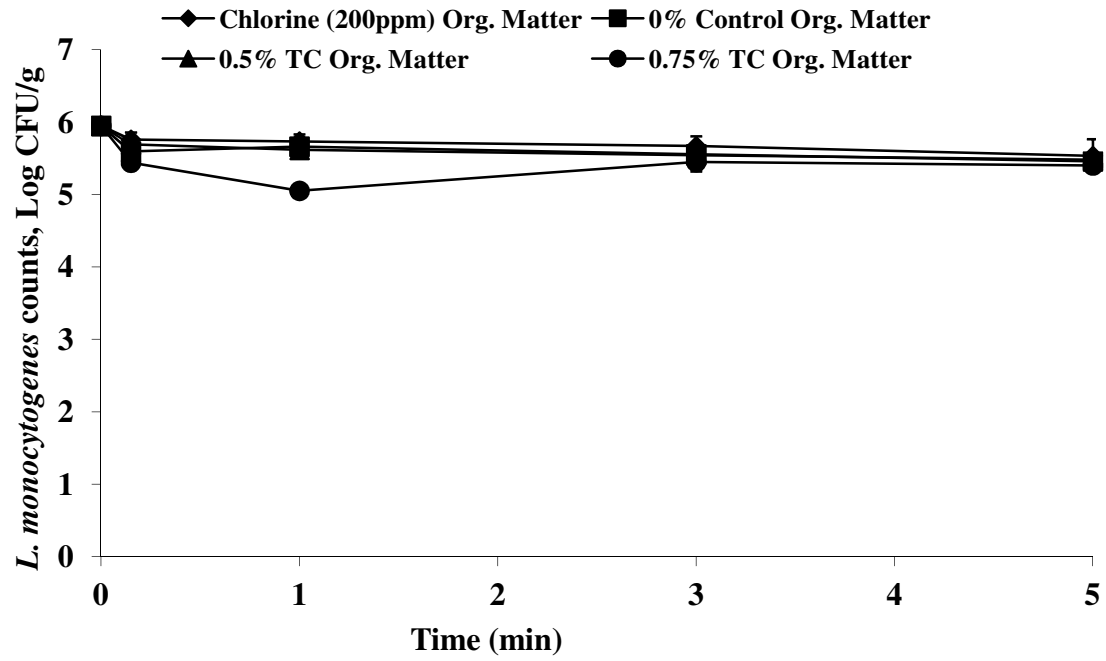


Fig. 8



Chapter 5: Conclusion

Four plant derived compounds, namely carvacrol (CAR), *trans*-cinnamaldehyde (TC), eugenol (EUG) and β -resorcylic acid (BR) were investigated as wash treatments for reducing *Salmonella* spp. on tomatoes. In addition, the efficacy of TC and CAR in inactivating *E. coli* O157:H7 and *L. monocytogenes* on iceberg lettuce in the presence and absence of 10% organic matter was studied. Results suggest that CAR, TC, EUG and BR were effective in rapidly reducing large populations of *Salmonella* spp. on tomatoes when used in wash water, and CAR rapidly decreased *E. coli* O157: H7 and *L. monocytogenes* populations on lettuce even in the presence of organic matter. The efficacy of aforementioned plant compounds for decontaminating fresh produce should be validated under commercial settings. In addition, extensive sensory and storage studies of fresh produce treated with plant compounds are warranted before recommending their usage in the produce industry.